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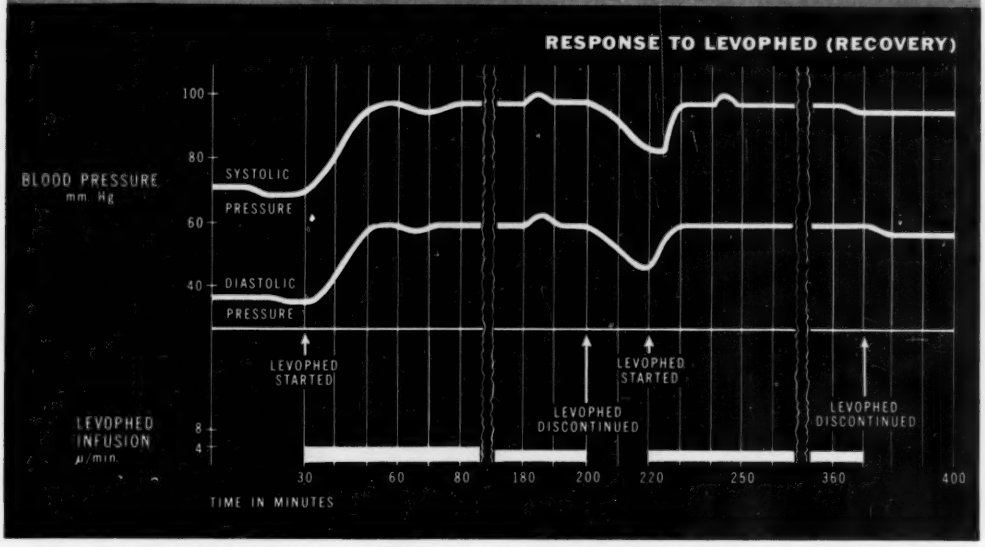

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References:

1. Kurland, G. S., and Malach, Monte: *New England Jour. Med.*, 247:383, Sept. 11, 1952.
2. Sayen, J. J., et al.: *Jour. Clin. Investigation*, 31:658, June, 1952.
3. Gilchrist, A. R.: *Brit. Med. Jour.*, 2:351, Aug. 16, 1952.
4. Miller, A. J., et al.: *J.A.M.A.*, 152:1198, July 25, 1953.
5. Levine, H. D., and Levine, S. A.: *Med. Clin. North America*, 37:955, July, 1953.

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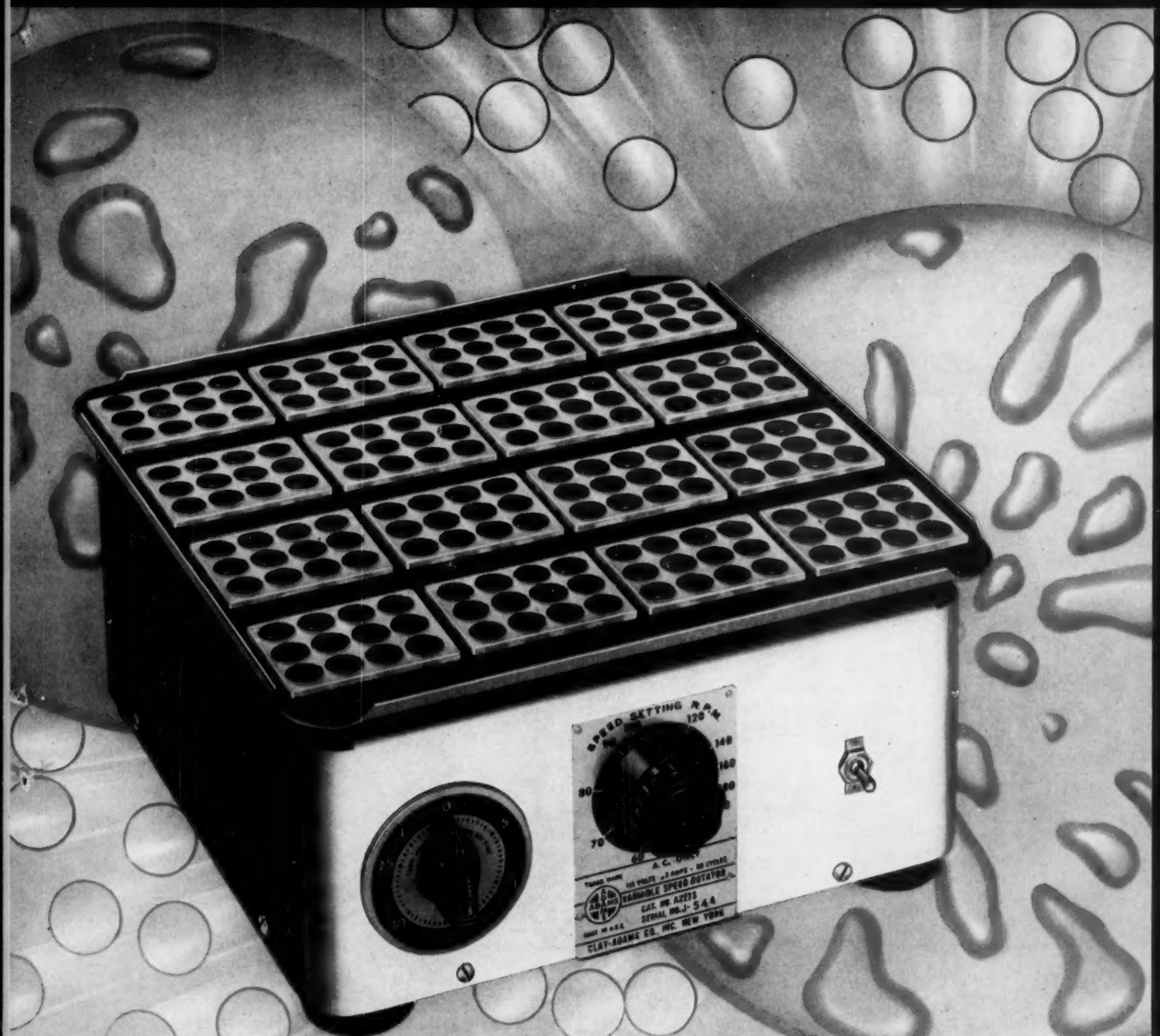

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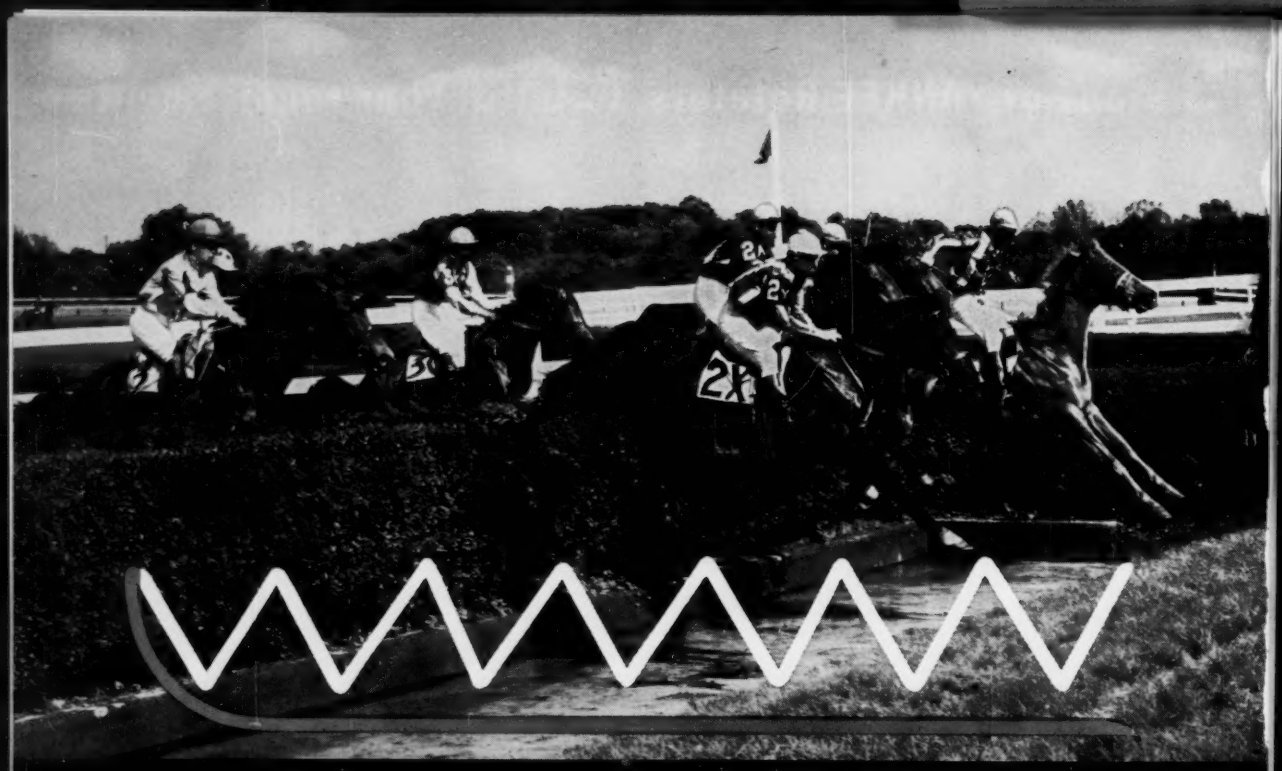

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THE ACTION OF CORTISONE AND DESOXYCORTICOSTERONE ACETATE ON GLOMERULAR FILTRATION RATE AND SODIUM AND WATER EXCHANGE IN THE ADRENALECTOMIZED DOG

By OLIVER GARROD,[1] S. A. DAVIES, and GEORGE CAHILL, Jr.

(From the College of Physicians and Surgeons, Columbia University, New York, N. Y.)

(Submitted for publication December 1, 1953; accepted February 2, 1955)

Adrenal failure causes a complex disturbance of renal function and of sodium and water exchange. In the dog, as in man, the ability to conserve sodium is impaired (1, 2), there is a fall in glomerular filtration rate and renal blood flow (3–6), and diuresis from water ingestion is much delayed (7, 8).

There have been many reports of the effects of corticosteroids on discrete renal functions such as filtration rate or excretion of water and electrolytes in adrenal insufficiency. There have, however, been few attempts to correlate the changes in these renal functions, one with another and with the level of bodily hydration. Thus, from existing data, the effects of changes in filtration rate on other renal functions, or of changes in bodily hydration on filtration rate, cannot be clearly defined in relation to the action of corticosteroids.

The steroids most examined for their renal effects have been desoxycorticosterone acetate (DCA) and, to a much lesser extent, cortisone. Though DCA appears to act mainly on electrolyte excretion, yet, when given in larger amounts than are needed for optimal sodium balance, it may then correct the low filtration rate of adrenalectomized dogs (4, 8, 9). It is not clear, however, whether this last effect is mainly or wholly dependent on excessive sodium retention, for no comparable studies appear to have been made at deliberately optimal levels of sodium balance (10, 11).

Cortisone seems to be more active than DCA in restoring filtration rate (12–14) and water diuresis (15, 16), and, by mechanisms which are not yet understood, it can either increase or diminish the sodium output (14, 15, 17–19). There is little information about its effects in the adrenalectomized dog (14, 20).

The investigations reported in this paper are an attempt to define more precisely the renal actions of cortisone and DCA, with special reference to their effects on filtration rate and on sodium and water exchange.

EXPERIMENTAL PLAN AND METHODS

Renal function was studied in two trained female mongrel dogs, before and after bilateral adrenalectomy, with the following objects: (a) to measure the effects of adrenalectomy on GFR and renal excretion of water and electrolytes, under controlled conditions of bodily hydration, but during escape from direct corticosteroid effects; (b) to compare the acute effects of cortisone and DCA on these renal functions, after adrenalectomy; and (c) to correlate the changes in these different renal functions with one another and with the state of bodily hydration.

Maintenance of the dogs

The dogs weighed 16 [2] and 20 Kgm. They lived under optimal conditions on a constant diet to which 2 gm. of sodium chloride was added.[3] They were fed between 1 and 3 P.M. and had free access to water. After a two-stage adrenalectomy, a state of optimal bodily hydration was maintained by daily intramuscular injection, at the time of each meal, of 0.6 to 0.75 mg. of DCA in sesame oil. In dog A changes in bodily hydration were induced by raising or lowering the DCA dosage (range, 0.5 to 1.5 mg. a day). Therapy was controlled by daily weighing and frequent measurements of fasting plasma specific gravity, electrolyte concentrations, blood urea, and hematocrit. Plasma volumes were measured by the T-1824 dye method (21), using a Beckmann spectrophotometer.

The diurnal patterns of water, creatinine, and electrolyte excretion were determined, in relation to meals and DCA injections.

[1] During tenure of a Rockefeller Travelling Fellowship in Medicine, 1951–1952. Present address: Institute of Clinical Research, The Middlesex Hospital, London, W. 1.

[2] Dog A was not fully grown at the beginning of these studies (initial weight, 15.5 Kgm.; final weight, 18 Kgm.).

[3] The total daily intake was approximately 90 mEq. sodium and 62 mEq. potassium.

Measurements of renal function

Usual procedure. After free access to water overnight, 15-minute urine collections were started between 8 and 9 A.M. Half an hour later, a priming infusion of creatinine and PAH[4] was slowly injected, followed by a sustaining infusion[4] which was continued throughout the experiment. A 45-minute period was allowed for equilibration. After three or more clearance periods, 40 ml. of warm water per Kgm. were given by stomach tube. Clearances were then measured for a further three to six hours.

The following renal functions were determined: GFR, ERPF, urinary pH, and urinary outputs of sodium, potassium, chloride, and phosphate.

Blood was taken initially and at the midpoint of every third or fourth clearance period, and the red cells reinfused at the end of each experiment. Plasma creatinine, PAH, specific gravity, and electrolyte concentrations were measured in each sample.

The above-mentioned procedure was carried out under the following conditions: (i) before adrenalectomy, (ii) after adrenalectomy, while on optimal DCA therapy, and, in dog A, also when on inadequate and excessive DCA therapy and after intravenous saline infusion, and in both dogs during acute administration of cortisone and DCA.

To observe its effects, both with and without a water load, cortisone was given, in separate experiments, three hours before the water dose, or at the time of the water dose, or three hours after it. The cortisone was given in one of three different ways: (i) intravenously, as a microcrystalline suspension of 50 mg. cortisone acetate in 1 ml. distilled water, dose 10 to 50 mg.[5]; (ii) intravenously, as cortisone tricarballylate,[6] dose 30 to 90 mg. freshly dissolved in a few ml. of isotonic saline, or (iii) orally, as cortisone acetate (Cortone®, Merck), dose 50 mg.

DCA was injected intravenously as a microcrystalline suspension in distilled water, made to the same specifications as the cortisone acetate,[5] dose 2.5 to 5.0 mg. It was given three hours after the water dose.

Other procedures. On 47 occasions the diuretic response to the standard water dose (supra) and the accompanying outputs of sodium and potassium were measured for three hours without determining GFR and ERPF.

In another group of experiments the effects of intravenous cortisone acetate, and of DCA, on excretion of water, electrolytes, and endogenous creatinine were measured but, without the complicating effects of an infusion or water dose.

Chemical methods

The methods used for the following chemical estimations are indicated by the references: sodium and potassium, by an internal lithium standard flame photometer (22) having an error of less than one per cent; plasma and urinary chloride (23); plasma and urinary creatinine (24); PAH (25); urinary phosphate (26); blood urea nitrogen (27); plasma specific gravity (28, 29). The urinary pH was measured by indicators within one-half minute of urine collection.

RESULTS

I. *Water and Electrolyte Balance in Relation to Chronic DCA Therapy After Adrenalectomy*

Assessment of bodily hydration

Adequacy of the DCA therapy was assessed by observing the weight, appetite, and activity

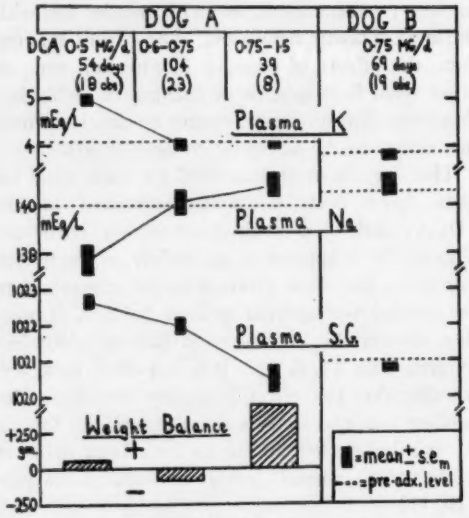

FIG. 1. THE EFFECTS OF DCA THERAPY ON FASTING PLASMA CONCENTRATIONS IN THE TWO ADRENALECTOMIZED DOGS

In dog A, optimal therapy was achieved on 0.6 to 0.75 mg. DCA a day, in dog B on 0.75 mg. a day. The weight balances in dog A are the means of daily deviations from the growth-weight curve during the total period of the studies. The slight rise in weight during inadequate DCA therapy was due to water retention, and the rise during excessive DCA therapy to salt and water retention.

[4] The priming infusion consisted of hypertonic creatinine and PAH in about 60 ml. of distilled water. The sustaining infusion consisted of approximately isotonic creatinine and PAH, and was given at a steady rate of 24 to 30 ml. per hour. The effects of these infusions on water and electrolyte excretion were measured during equilibration, and on GFR and ERPF during succeeding periods totalling 45 to 60 minutes, before the water dose was given.

[5] Specially prepared by Chemical Specialities, Inc., N. Y.

[6] A water-soluble but unstable ester, specially prepared by Merck, Ltd., N. J.

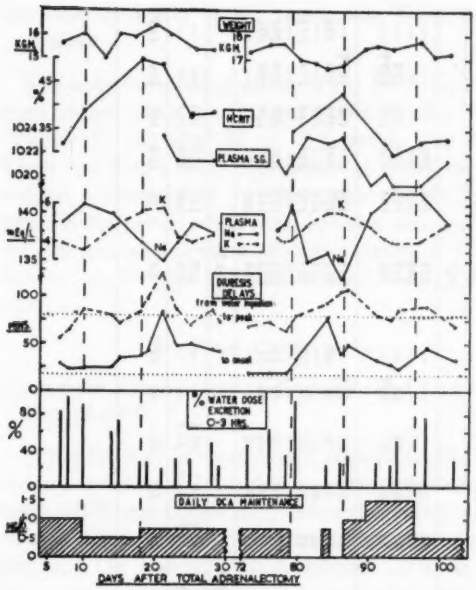

FIG. 2. WATER DIURESIS AND PLASMA CONCENTRATIONS RELATIVE TO DCA THERAPY IN ADRENALECTOMIZED DOG A

Two sample periods are shown. Diet and sodium intake were constant. The body weight and plasma concentrations were obtained after 18 hours' fasting with free access to water, before giving the standard water dose of 40 ml. per Kgm. body weight. Dotted lines indicate the delay in diuresis before adrenalectomy. The body weights have not been corrected for growth.

of the dogs, and by comparing their fasting plasma specific gravities and electrolyte concentrations with the levels obtained before adrenalectomy (Figure 1). In dog A, a state of optimal health and bodily hydration was achieved on 0.6 to 0.75 mg. DCA per day, in dog B on 0.75 mg. per day. At this dosage their plasma volumes varied between 5 and 5.7 per cent of body weight, figures comparable with the mean of 5.4 per cent reported by Courtice in 29 intact mongrel dogs (30). In dog A the dose of DCA was deliberately varied so that the effects of changes in bodily hydration could be studied. Inadequate DCA therapy (*e.g.*, 0.5 to 0.6 mg.) was followed by a rise in hematocrit, plasma specific gravity and potassium and a fall in plasma sodium; the expected loss of weight did not always occur (Figure 1) because of water retention, apparently intracellular, from the repeated water doses (Figure 2). Excessive DCA therapy (*e.g.*, 1 to 1.5 mg.) caused excessive gain in weight, a fall in plasma specific gravity, and a slight rise in plasma sodium concentration (Figures 1 and 2). The plasma specific gravity was the most sensitive index of hydration; the hematocrit and plasma sodium and potassium were almost as sensitive to inadequate, but not to excessive, therapy; blood urea was an unreliable index.

In order that renal function could be assessed in relation to bodily hydration, the renal data were grouped as follows: 1) optimal therapy, all periods on 0.6 to 0.75 mg. DCA per day,[7] 2) inadequate therapy all periods on less than 0.6 mg. per day, 3) excessive therapy, all periods on 1 to 1.5 mg. per day, and in dog A, on 0.75 mg. for more than six weeks.[7]

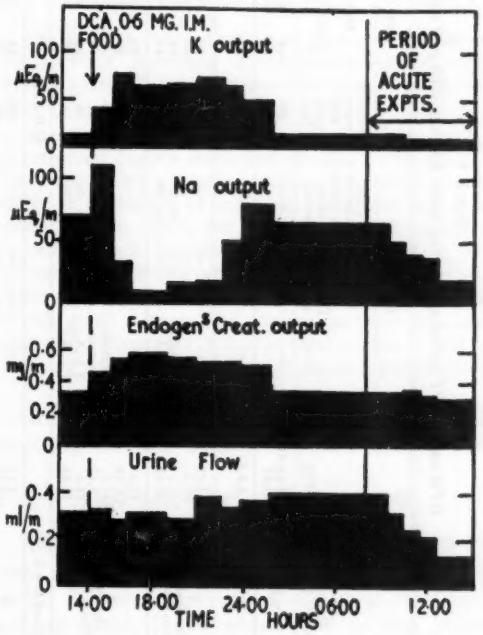

FIG. 3. DIURNAL VARIATIONS IN URINARY EXCRETION RATES DEPENDENT ON FEEDING AND DCA THERAPY, IN ADRENALECTOMIZED DOG A

Free access to water was allowed throughout the 24-hour period. The pattern of urinary excretion was similar on other occasions, and in both dogs. Note the transient effect of the DCA injection.

[7] In dog A clear signs of overdosage appeared after six weeks on 0.75 mg. DCA per day. The optimal dosage was probably nearer 0.6 than 0.75 mg. per day.

TABLE I

GFR and ERPF before and during water diuresis, with and without cortisone *

	Adrenal status	DCA therapy (daily dose)	Date	Cortisone: Ester	Cortisone: Dose mg.	Cortisone: Route	Cortisone: Time relative to water dose mins.	GFR (ml./min.): Before† water and cortisone	GFR: Hrs. after water dose‡ 1	2	3	4	5	ERPF (ml./min.): Before† water and cortisone	ERPF: Hrs. after water dose‡ 1	2	3	4	5
	Intact	Nil	XII.14.51	—	—	—	—	65	64	63	61	—	—	—	—	—	—	—	—
			XII.17	—	—	—	—	64	62	64	—	—	—	—	—	—	—	—	—
	Unilateral adrenalectomy	Nil	I.4.52	—	—	—	—	50	52	55	55	—	—	159	187	145	155	—	—
Dog A	Total adrenalectomy	A Inadequate (0 to 0.5 mg.)	I.25	—	—	—	—	37	49	47	45	—	—	120	144	140	141	—	—
			IV.3	—	—	—	—	36	40	45	45	—	—	91	89	127	129	—	—
			V.2	Acetate	23	i.v.	+ 15	33	44	*52*	*51*	*50*	*56*	90	108	*121*	*107*	*128*	*135*
			V.15	T.C.A.‖	30	i.v.	+ 30	39	44	*46*	*45*	*44*	*41*	114	138	*139*	*139*	*148*	*142*
		B Optimal (0.6 to 0.75 mg.)	II.27	—	—	—	—	38	39	43	42	44	42	110	114	131	139	151	129
			IV.24	—	—	—	—	32	39	44	48	—	—	102	126	153	148	—	—
			III.7	Acetate	23	i.v.	+ 30	40	38	*52*	*50*	*48*	*48*	155	156	*324*	*256*	*180*	—
			VI.17	Acetate	50	oral	−180	41	*54*	*58*	*53*	*54*¶	—	117	*130*	*160*	*179*	*174*¶	—
			II.8	T.C.A.‖	30	i.v.	+ 30	36	44	*46*	*48*	*51*	*52*	106	127	*135*	*136*	*155*	*163*
			II.18	T.C.A.‖	30	i.v.	+ 30	38	41	*44*	*44*	*49*	*52*	100	105	*123*	*131*	*143*	*150*
		Mean A+B						37.0±2.8	—					111±18					
		C Excessive (0.75 to 1.5 mg.)	I.17	—	—	—	—	54	55	61	61	—	—	160	161	175	165	—	—
			III.27	—	—	—	—	42	52	62	61	60	57	119	152	188	179	166	176
			IV.14	—	—	—	—	48	52	57	58	57	—	129	137	157	163	170	—
			III.18	Acetate	23	i.v.	−180	51	*75*	*70*	*64*	—	—	103	*278*	—	—	—	—
		Mean C						48.7						128					
	Intact	Pregnant	II.14.51	—	—	—	—	77	86	81	—	—	—	255	384	296	—	—	—
			II.25	—	—	—	—	74	82	74	—	—	—	205	262	266	—	—	—
		6 to 7 weeks after delivery	IV.30	—	—	—	—	51	52	61	65	61	—	135	145	199	197	174	—
			V.9	—	—	—	—	48	52	64	57	54¶	—	119	168	221	173	159¶	—
Dog B	Total adrenalectomy	D Optimal (0.75 mg.)	VI.3	—	—	—	—	47	55	58	57	51¶	—	154	174	185	196	184¶	—
			VI.12	—	—	—	—	47	51	56	56	57	60	162	187	200	201	209	223
			VI.19	Acetate	50	oral	−180	47	*62*	*64*	*62*	—	—	171	*178*	*213*	*228*	—	—
			VI.24	Acetate	23	i.v.	+ 30	37	42	*53*	*52*	*57*	*53*	128	*141*	*205*	*186*	*214*	*213*
			VII.1	—	—	—	—	51	—	—	—	—	—	139	—	—	—	—	—
			VII.10	Acetate	50	i.v.	− 30	43	*52*	*58*	*59*	*56*	*55*	154	*176*	*202*	*207*	*199*	*203*
			VII.15	—	—	—	—	44	42	50	52	54	51	132	144	170	170	200	209
			VII.30§	—	—	—	—	39	48	50	46	—	—	154	202	217	157	—	—
		Mean D						44.4±6.9						149±18					
Both Dogs	Total adrenalectomy	Deficient + Optimal	Mean, without cortisone (8 expts.)					40	45.4	49.1	48.8	—	—	128	147	165	160	—	—
			Mean, cortisone acetate (6 expts.)					40.1	*48.7*	*56.1*	*54.5*	—	—	136	*148*	*204*	*194*	—	—
Dog A	Total adrenalectomy	Optimal	VII.18	After i.v. isotonic saline infusion, 40 ml./Kgm.				48	41	50	45	60	64**	88	84	114	107	129	143**

* Figures in italic type refer to data after cortisone.
† Measurement at 45 to 60 minutes after start of sustaining infusion.
‡ Each hourly value is the mean of four consecutive 15-minute measurements.
§ Three mg. DCA per day for three days.
‖ Tricarballylate.
¶ Forty-five min. periods.
** Thirty min. periods.

Duration of action of the therapeutic DCA injections

The sodium-retaining action of the DCA injections ceased after about eight hours in dog A (Figure 3) and after 12 to 18 hours in dog B. There was then a delayed excretion of the dietary sodium. From 18 to 24 hours after the DCA, which is the period when renal function was studied, the sodium excretion remained steady or fell further; the kidneys had then escaped from DCA effect.

II. *Renal Function*

Glomerular filtration rate (GFR) and renal plasma flow (ERPF) [8]

(a) *Effects of adrenalectomy, relative to DCA therapy (Table I).* In dog A, after adrenalectomy, GFR was consistently subnormal when on optimal DCA therapy, but rose after water ingestion. On excessive DCA therapy GFR was higher and sometimes became normal after water ingestion. Changes in ERPF were essentially parallel.

In dog B, GFR fell to subnormal levels during the second month after adrenalectomy, but throughout the post-operative period it rose less after water than before adrenalectomy. A short period of excessive DCA therapy in this dog (3 mg. a day for three days) did not raise the GFR (Table I), but there was no accompanying retention of sodium and water.

(b) *Acute effects of DCA on GFR.* Intravenous DCA (2.5 to 5 mg.), though it caused almost maximal reabsorption of sodium by the tubules, did not increase GFR, ERPF, or endogenous creatinine excretion, or augment the effect of cortisone on these functions (Figure 4, Table II).

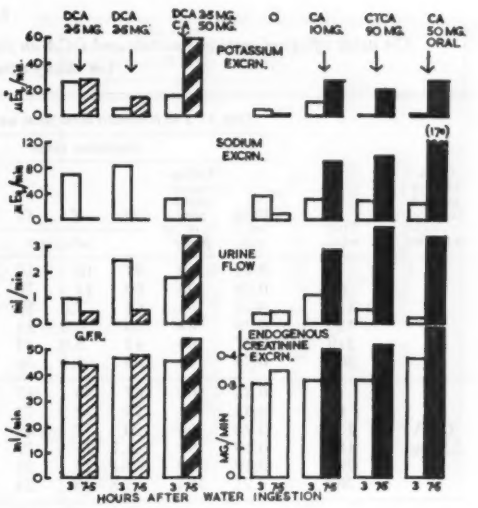

FIG. 4. THE ACUTE EFFECTS OF DCA AND CORTISONE ON URINARY EXCRETION RATES WHEN GIVEN THREE HOURS AFTER WATER INGESTION, IN ADRENALECTOMIZED DOG A

Ingestion of the standard water dose was followed by a subnormal diuresis which was subsiding at three hours when the steroid was given. Cortisone caused a rise in GFR or endogenous creatinine excretion and return of water diuresis. DCA had no effect on GFR or water diuresis. Each excretion rate shown covers a 45-minute period ending at three, or seven and one-half hours after water ingestion, as indicated at the bottom of the graph. After DCA, sodium excretion was less than 4 μEq. per minute. CA = cortisone acetate, CTCA = cortisone tricarballylate, given I.V. except when specified.

(c) *Acute effects of cortisone on GFR.*

(i) *Without water ingestion.* In both dogs 50 mg. of intravenous cortisone acetate caused a mean 30 per cent rise in endogenous creatinine output which began within one to two hours (Table II). That this was due to the acute rise in GFR was confirmed in dog A; within three and one-half hours of intravenous injection of 23 mg. of cortisone acetate GFR had risen from 53 to 68 ml. per minute, and ERPF from 116 to 222 ml. per minute (Table III).

(ii) *Accompanying, or preceding, water ingestion.* The rise in GFR and ERPF from water ingestion was greater with cortisone than

[8] The effects on GFR and ERPF of the infusions used in measuring them need to be considered. During nine experiments in dog A, there was a consistent rise in GFR of 11 ± s.d. 3.7 ml. per minute, and in ERPF of 8 ± s.d. 2 ml. per min. between 45 and 90 minutes after starting the sustaining infusion. Though a similar rise did not occur in dog B, a rise in this dog's sodium excretion within 30 mins. of starting the sustaining infusion suggested that GFR had already risen during the period of equilibration when it could not be measured directly. Thus, in both dogs the true fasting GFR was probably less than that of the first period of creatinine clearance at the end of equilibration. To ensure standardization, the fasting GFR and ERPF have been taken from the first 15-minute clearances after equilibration, *i.e.*, 45 to 60 minutes after starting the sustaining infusion.

TABLE II

The acute effects of cortisone acetate and DCA on fasting urinary excretion rates in two adrenalectomized dogs (on steady optimal DCA therapy) *

Intravenous steroid (microcrystals) at 0 mins.	Time mins.	Dog A: 5 to 6 months after total adrenalectomy — Excretion rates: Urine ml./min.	Endogenous creatinine % of 1st period	Na μEq./min.	K μEq./min.	Cl μEq./min.	PO4† μEq./min.	Urine pH‡	Dog B: 8 to 11 weeks after total adrenalectomy — Excretion rates: Urine ml./min.	Endogenous creatinine % of 1st period	Na μEq./min.	K μEq./min.	Cl μEq./min.	PO4† μEq./min.	Urine‡ pH
	0	0.41	100	94	16	77	27	7.5	0.25	100	37	21	27	13	7.5
	60	0.39	98	89	13	78	22	7.5	0.29	97	55	14	30	12	7.8
0	120	0.32	93	62	7.6	59	12	7.45	0.27	100	49	10.6	31	9.5	7.8
	180	0.28	88	56	6.5	51	10	7.5	0.30	101	58	9.1	31	8.1	7.8
	240	0.27	90	42	5.9	39	7.5	7.55	0.26	103	46	8.2	32	6.1	7.8
	300	0.24	91	35	4.4	34	6.1	7.6	0.28	99	48	7.3	34	5.2	7.7
	0	0.45	100	87	30	87	27	7.4	0.36	100	91	12	70	7.4	8.0
	60	0.38	91	73	22	72	25	7.1	0.35	99	79	8	92	4.6	8.2
DCA	120	0.28	90	20	37	49	17	6.7	0.23	91	32	21	54	2.9	7.4
2.5 mg.	180	0.19	92	4.7	23	27	12	6.8	0.26	102	31	27	57	4.3	7.3
	240	0.19	99	3.1	23	26	13	7.1	0.25	108	33	27	61	9.1	7.4
	300	0.21	104	2.8	26	24	16.5	7.3	0.25	104	29	16.2	45	7.1	7.3
		(mean of 3 experiments)							(mean of 2 experiments)						
	0	0.40	100	73	16.2	60	31	7.2	0.35	100	85	9.1	78	14	7.5
Cortisone	60	0.37	98	90	33	101	23	7.6	0.36	107	83	22	73	11	8.1
acetate	120	0.29	103	33	39	50	12	7.25	0.77	120	154	52	176	11	7.9
50 mg.	180	0.72	115	24	47	48	19	7.3	0.54	124	109	46	133	9.7	7.5
	240	0.86	128	89	56	61	34	8.0	0.76	129	119	35	118	16	7.8
	300	0.91	128	146	47	53	36	8.25	0.83	132	145	17	78	27	8.2

* Each hourly output is the mean of two consecutive one-half-hourly outputs. Plasma changes are shown in Table IV
† Corrected for urine pH.
‡ Measured by indicators within 30 seconds of urine collection.

TABLE III

The effect of intravenous cortisone on renal function and water balance in adrenalectomized dog A *

Time mins.	Urine flow ml./min.	GFR ml./min.	ERPF ml./min.	Excretion rates μEq./min. Na	K	Cl	Cumulative water balance (ml.) Uncorrected: Water infused (W)	Urine vol. (UV)	Total water balance (W−UV)	Corrected for Na loss: Urin. water in excess of isotonic sodium (UW)	Corrected water balance (W−UW)	Time mins.	Plasma concentrations: S.G.	Na mEq./L.	K mEq./L.
0–15	.58			61	17	55	0	10	− 10						
16	Start of priming infusion (creatinine and PAH in 58 ml. distilled water)														
25	Start of sustaining infusion, continued at 30 ml./hr. throughout experiment														
55	.93	—	—	76	16	53	82	69	+ 13						
70	.68	51	103	95	12	65	90	80	+ 10	32	+ 58	63	1.0205	—	—
85	.69	53	109	156	17	76	97	89	+ 8	26	+ 71				
100	.69	52	114	139	18	75	105	98	+ 7	22	+ 83				
115	.69	57	137	135	17	80	112	108	+ 4	18	+ 94	108	1.0205	143	3.8
117	23 mg. cortisone acetate microcrystals intravenously														
160	.75	53	141	125	19	94	134	142	− 8	15	+119	153	1.0205	143	3.7
205	3.19	62	170	252	83	299	155	288	−133	85	+ 70	198	1.0215	143	3.5
250	1.37	66	183	93	65	117	177	350	−173	119	+ 58	243	1.0215	140	3.2
295	2.16	64	187	68	40	57	198	456	−258	205	− 7	288	1.0220	141	3.2
340	2.14	68	222	149	45	71	220	562	−342	246	− 26	333	1.0215	142	3.0

* The dog was on excessive DCA maintenance (0.75 mg. per day for seven weeks).
Each output shown after cortisone injection is the mean of three consecutive 15-minute observations.

without (Table I). This increment occurred at about one and one-half hours after 23 mg. of cortisone acetate, and at three to three and one-half hours after an equimolar dose (*i.e.*, 30 mg.) of cortisone tricarballylate, irrespective of the level of DCA therapy (Table I).

(iii) *Three hours after water ingestion* (*Figure 4*). When cortisone was given three hours after the water dose, orally as the acetate, or intravenously as the acetate or tricarballylate, endogenous creatinine excretion rose by 25 to 33 per cent. Without cortisone it did not rise.

(d) *GFR relative to plasma volume and specific gravity.* Plasma specific gravity was used as an index of hemodilution and was measured concurrently with GFR during all experiments. Grouped data from the periods without cortisone showed a high inverse correlation between GFR and plasma specific gravity ($r = -0.84$). Grouped data from the periods between one and six hours after cortisone showed no such correlation ($r = -.034$), but GFR was higher relative to plasma specific gravity (Figure 5).

Again, in four experiments unaccompanied by infusion or water ingestion, 50 mg. of intravenous cortisone acetate had no effect during four hours on plasma volume, specific gravity or electrolyte concentrations; concurrently, however, the en-

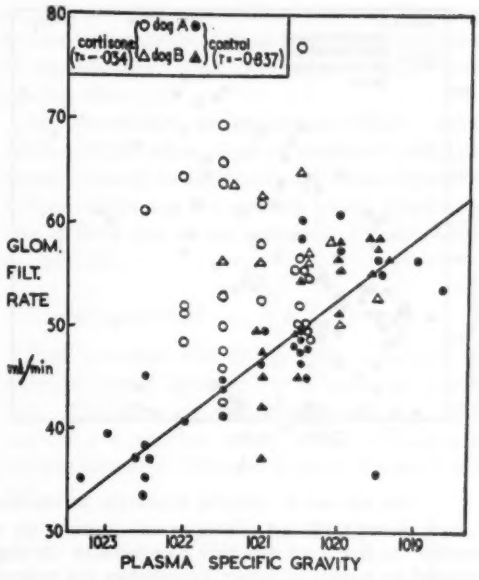

FIG. 5. GFR IN RELATION TO PLASMA SPECIFIC GRAVITY, WITH AND WITHOUT CORTISONE, IN TWO ADRENALECTOMIZED DOGS

Each GFR plotted is the mean of three consecutive 15-minute measurements. Plasma specific gravity (28) was measured at the midpoint of each 45-minute GFR period. All data have been included except those from the first two months after adrenalectomy in dog A.

TABLE IV

Changes in plasma in relation to changes in endogenous creatinine excretion after cortisone and DCA *

Subject	Steroid at 0 mins. (Intravenous)	Per cent change at three and three-fourths hours after steroid: Endogenous creatinine output†	Plasma‡: Vol.	S.G.§	Na conc.	Cl conc.
	Cortisone 50 mg.	+33	− 4.5	−1.3	+0.4	+1.6
Dog A	50 mg.	+36	− 1.4	+1.6	−0.6	−0.5
	50 mg.	+21	—	+1.7	−1.4	—
Dog B	50 mg.	+27	+12.7	+0.4	−0.2	+0.4
	50 mg.	+36	− 2.9	+1.0	−0.5	−0.2
Mean of 5 experiments (both dogs)		+30.6	+ 1.0	+0.7	−0.46	+0.32
Dog A	DCA 2.5 mg.	− 2	—	−0.5	+0.7	+2.1
Dog B	DCA 2.5 mg.	+ 1	—	+0.9	+1.0	0
Dog A	0	−10	—	−1.3	−1.0	0
Dog B	0	+ 3	—	—	—	—

* These experiments were made during a five-week period of steady optimal DCA therapy, after 18 hours' fasting with free access to water.

† Calculated from the output three and one-half to four hours after, the steroid injection divided by the output during the 60 minutes immediately preceding, the steroid injection.

‡ These measurements were made 15 to 30 minutes before, and repeated three and three-fourths hours after, the steroid injection.

§ Measured by the gradient method of Lowry and Hunter (29).

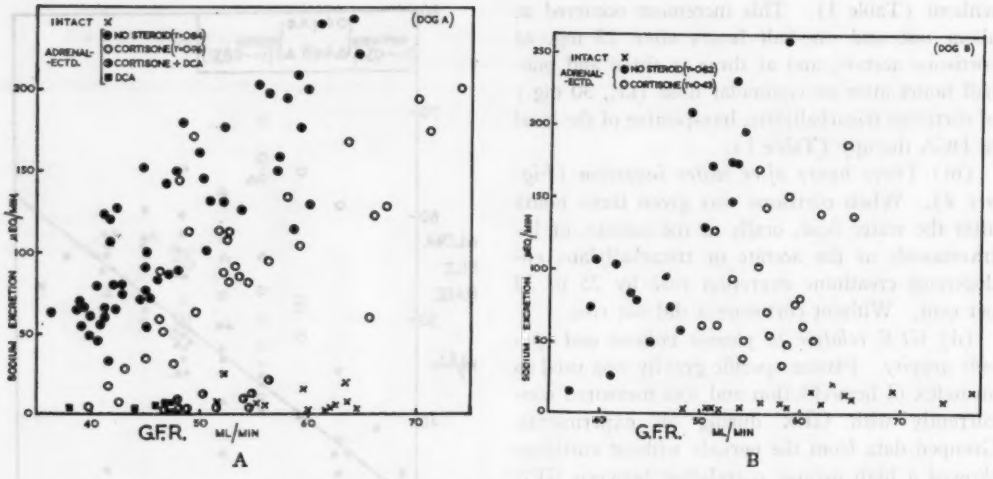

FIG. 6A AND B. SODIUM EXCRETION IN RELATION TO GFR: THE EFFECTS OF CORTISONE AND DCA

Each plotted GFR and sodium excretion rate is the mean of three consecutive 15-minute measurements, without overlap. In dog A all data have been included. In dog B, data from the first month after adrenalectomy have been excluded on account of dietary irregularities and unsteady sodium intake.

dogenous creatinine output rose by 30 per cent (Table IV). These findings suggested that the acute effects of cortisone on GFR were not dependent on an increase in plasma volume.

(e) *The effect of saline infusion on GFR (Table I).* This experiment was designed to find out whether the chronic effects of excessive DCA therapy on GFR could be reproduced acutely, when on optimal DCA therapy, by infusing isotonic saline. Isotonic saline, 40 ml. per Kgm. of body weight, was infused during 75 minutes. When the water dose was given at the end of this infusion, GFR was 10 ml. higher than the mean fasting level on optimal DCA therapy (37.1 ± s.d. 2.8 ml. per min.). Four hours later GFR had risen to its pre-adrenalectomized level (Table I). This effect was preceded by defecation, slight edema, and restlessness, suggesting mild water intoxication. Thus, the chronic effects of excessive DCA therapy on GFR were reproduced acutely by overloading with sodium and water; water diuresis, however, was largely inhibited.[9]

[9] Defecation during diuresis experiments in the intact dog is usually accompanied by prolonged antidiuresis (31). This was also our experience on other occasions, in the adrenalectomized dog.

Sodium excretion [10]

(a) ***In relation to water diuresis.*** Before adrenalectomy, even when GFR rose after water ingestion, the fasting sodium output remained low or fell further (Figure 6). After adrenalectomy, water ingestion always raised GFR (Table I), and there was then a parallel rise in the fasting sodium excretion, an effect which was further accentuated when on excessive DCA therapy [10] (Figure 6).

(b) ***In relation to GFR, without cortisone.*** On analysis of grouped data from the periods after adrenalectomy, there was a high correlation between GFR and sodium output ($r = 0.84$ and 0.82 in the two dogs) (Figure 6).[11]

[10] Before adrenalectomy the sodium output, 18 hours after food, was always less than 25 μEq. per minute. After adrenalectomy it was always higher (40 to 100 μEq. per minute), due partly to delayed excretion of dietary sodium which had been retained during the period of action of the injected DCA. This "escape effect" was much greater during excessive DCA therapy, owing to the longer action of the larger DCA injections and the compensating effect of higher GFR (infra).

[11] Because the changes in plasma sodium concentration were small, when this data was calculated as the quantity of sodium filtered ($Na^F = Na^P \times GFR$) and the percentage reabsorbed by the tubules (Na R/F), the correlations were essentially similar.

(c) *In relation to GFR, after cortisone.* By raising GFR cortisone increased the sodium output. There was still a correlation between GFR and sodium excretion ($r = 0.77$ and 0.43 in the two dogs), but for any given level of GFR the rate of sodium excretion was less than when no steroid had been given (Figure 6).[11] Figure 7 shows typical changes in sodium filtration (Na^F) and reabsorption (Na R/F) after cortisone. It will be seen that there was an early and persistent rise in (Na^F). The fall in sodium reabsorption (Na R/F) was, however, interrupted at two to three hours, when cortisone was acting maximally on sodium reabsorption. After oral cortisone (adjoining graph) the rise in GFR was delayed, and until this rise occurred sodium reabsorption remained almost complete.

Thus, cortisone had both vascular (GFR) and metabolic (tubular sodium reabsorption) effects on the kidney, which did not necessarily coincide in their points of maximal intensity. In acute experiments the net result, which was increased sodium output, seemed to be due to preponderance of the filtration effect over the relatively weak tubular effect.

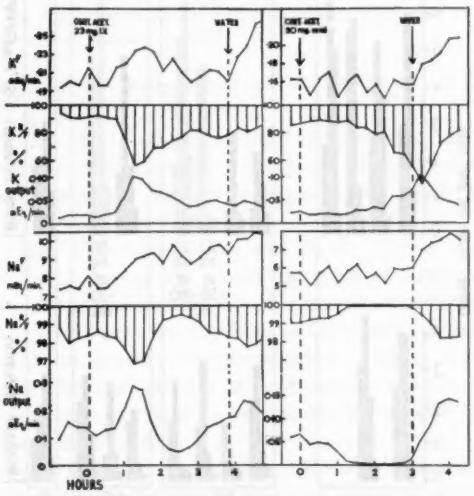

FIG. 7. THE EFFECT OF CORTISONE ON RENAL SODIUM AND POTASSIUM FILTRATION AND REABSORPTION, IN ADRENALECTOMIZED DOG A (TWO EXPERIMENTS)

The first experiment shows typical changes after intravenous cortisone. Independent of Na^F, tubular sodium reabsorption was greatest at about two and one-half hours. In the second experiment, oral cortisone did not raise Na^F acutely. Tubular sodium reabsorption was therefore complete until Na^F rose after water ingestion. Changes in potassium excretion were largely independent of GFR.

(d) *In relation to intravenous DCA.* Intravenous DCA, either alone or combined with cortisone, caused maximal tubular reabsorption of sodium within one and one-half hours, despite any rise in GFR due to the cortisone (Figures 4 and 6, Table II).

Potassium excretion

Both cortisone and DCA, given separately or together, caused an early rise in potassium excretion. This effect was largely independent of GFR and did not correlate closely with the changes in sodium excretion (Figures 4 and 7, Tables II and III).

Anion excretion (Table II)

During the first three hours after cortisone, the chloride excretion varied directly with the sum of the sodium and potassium excretion. During the next three hours this relationship ceased; chloride excretion then fell or remained steady, despite a further rise in cation excretion. Although the phosphate output always rose during the second three-hour period, by the fifth hour after cortisone the combined outputs of sodium and potassium exceeded those of chloride and phosphate by 60 to 100 μEq. per minute. Because the urine was then of maximal alkalinity,[12] most of this undetermined anion was probably bicarbonate. These changes did not occur after intravenous DCA.

Diuresis after water ingestion[13]

(a) *Renal aspects of diuresis after adrenalectomy.*

(i) *The effects of chronic DCA therapy (Figure 8).* Diuresis from water ingestion varied directly with the current level of DCA therapy.

[12] The possibility that urinary infection might have contributed to the abnormally high urinary pH was not excluded.

[13] Diuresis from the standard water dose was always greater when there was an accompanying infusion of creatinine and PAH. The two groups of experiments have therefore been shown separately in Figure 8.

Compared with the pre-adrenalectomized state,[14] during optimal DCA therapy both the onset and peak of diuresis were delayed, and the maximal rate of urinary flow and the percentage of the water dose excreted in three hours were both greatly diminished. During inadequate DCA therapy there were further delays in diuresis (Figure 2).

Excessive DCA therapy restored the pattern of diuresis almost to normal, as judged only by the urine volume. However, volume alone was not a true measure of water diuresis because of the high urinary sodium outputs (infra). In dog B, the delay in onset of diuresis was shortened by giving four times the optimal DCA therapy for three days, but the percentage of the water dose excreted in three hours was not increased (Figure 8). In this instance the higher DCA dosage was not continued for long enough to cause any gain in weight, hemodilution or elevation of GFR (Table I).

(ii) *Acute effects of cortisone* (*Figure 8*). Cortisone increased water diuresis above the control (adrenalectomized) volumes at each level of DCA therapy. After intravenous cortisone acetate this effect was seen within 60 to 90 minutes. Cortisone tricarballylate, somewhat slower in its action, augmented water diuresis before it raised GFR. In general, this effect of cortisone

[14] During the initial training period before adrenalectomy, at first diuresis was sometimes delayed and of poor volume. Because these failures seemed to be due to liberation of antidiuretic hormone, in response to catheterization, venepuncture, *etc.*, data from these early experiments have not been included in this paper.

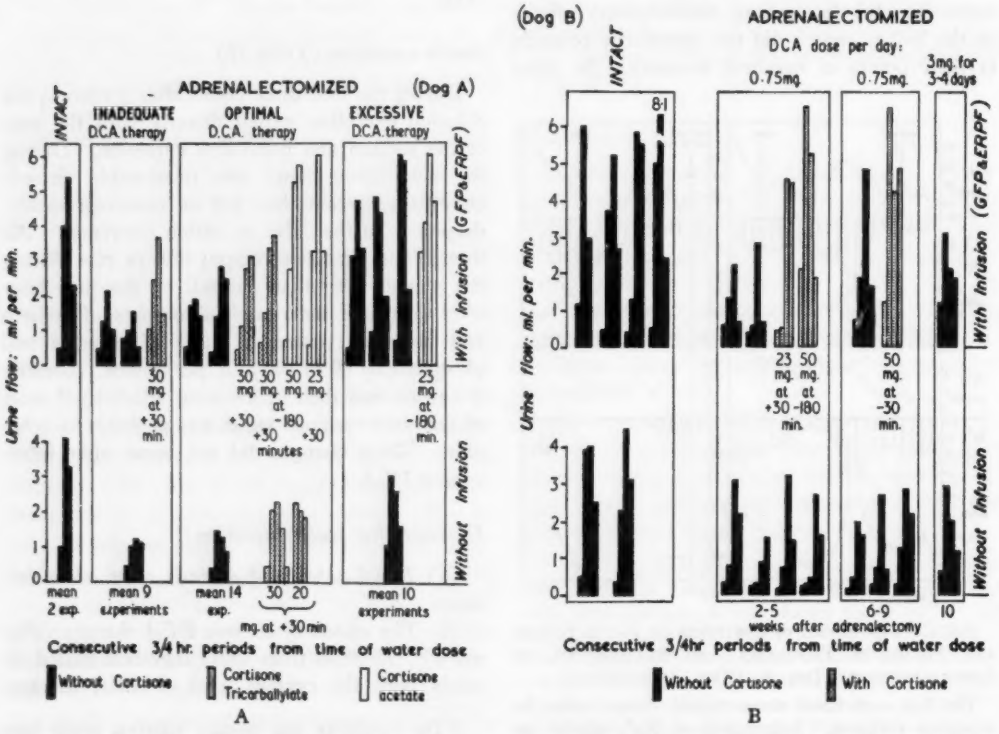

FIG. 8A AND B. THE EFFECTS OF ADRENALECTOMY, WITH AND WITHOUT CORTISONE, ON ACUTE DIURESIS FROM INGESTED WATER

A standard water dose of 40 ml. per Kgm. was given in all experiments. Because infusion of creatinine and PAH facilitated diuresis, the experiments in which GFR and ERPF were measured are shown separately. Figures below the data from cortisone experiments indicate the dose of cortisone, intravenous unless specified, and the time of its administration relative to the water dose.

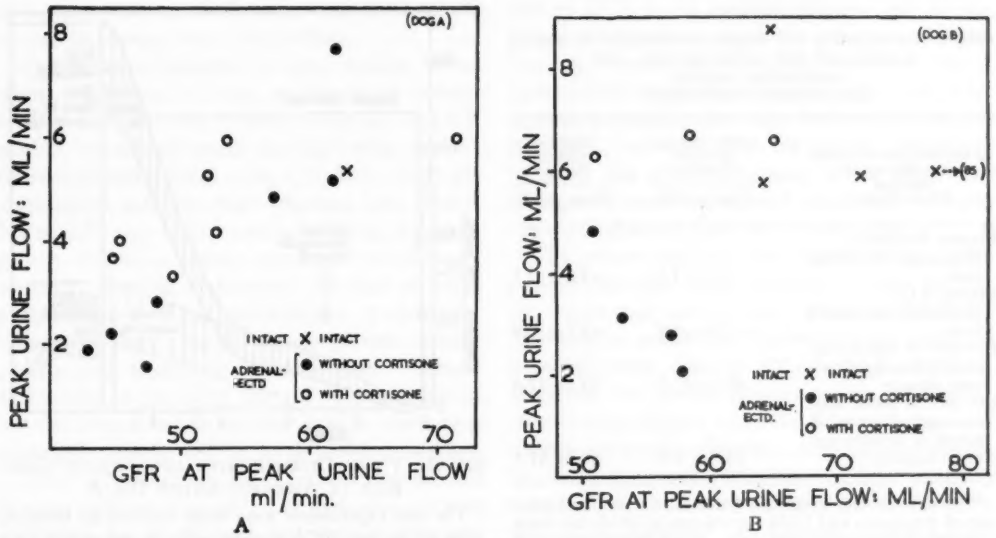

FIG. 9A AND B. MAXIMAL URINE FLOW RELATIVE TO GFR DURING DIURESIS IN TWO ADRENALECTOMIZED DOGS, WITH AND WITHOUT CORTISONE

All data plotted are the means of three consecutive 15-minute measurements, embracing the peak urine flow. In dog B there was no relation between GFR and maximal urine flow. In both dogs cortisone increased maximal urine flow relative to GFR.

was largely independent of the level of GFR (Figure 9).

(iii) *Acute effects of DCA* (*Figure 4*). Intravenous injection of 2.5 mg. of DCA did not increase water diuresis or augment the effect of cortisone on water diuresis.

(iv) *Urine osmolarity and GFR.* Urine osmolarity during the 45-minute peak of diuresis was calculated indirectly.[15] No consistent relation was found between the level of GFR and the capacity to secrete a hypotonic urine. During diuresis after cortisone the urine osmolarity was much lower than in control experiments, irrespective of the level of GFR.

(b) *Homeostatic aspects of water diuresis.* The function of water diuresis is to correct acutely, by selective excretion of water, the dilution of body fluids which results from water ingestion. After adrenalectomy, though water ingestion caused normal hemodilution,[16] the water loads were not excreted with normal rapidity, and the hemodilution persisted. These failures were acutely corrected by cortisone. The dilution of body fluids after water ingestion was measured by the falls in plasma specific gravity and sodium concentration. The return of these indices towards normal was then followed at intervals in relation to the total water excreted in excess of urinary isotonic sodium (Table V). In seven control experiments in the two adrenalectomized dogs, excluding the periods on excessive DCA therapy, at the end of three and three-fourths hours only 22 per cent of the water load had been excreted in this unobligated form and the plasma sodium concentration was still 5 per cent less than its initial value. In eight similar experiments with cortisone the findings, respectively, were 61 per cent excretion of water unobligated to sodium and 1.3 per cent reduction of the plasma sodium concentration. When the diuresis induced by cortisone after water ingestion was followed to its natural termination, the total urinary volume usually greatly exceeded the water load; yet, the total volume of urinary water unobligated to sodium, though sometimes equalling the water load,

[15] Calculated from the urinary concentrations of urea, creatinine, and (sodium and potassium × 2).

[16] The speed and magnitude of the fall in plasma specific gravity after water ingestion was the same after adrenalectomy as before. Water absorption from the gut was therefore presumed to be normal.

TABLE V

Plasma specific gravity and sodium concentration in relation to total water and sodium excretion, with and without cortisone (two adrenalectomized dogs) *

At three and three-fourths hours after water ingestion (*40 ml./Kgm.*)	Water without cortisone (7 expts.) Mean ± S.D. %	Water + cortisone (8 expts.) Mean ± S.D. %
Plasma Na conc.: % change from initial level	−5± 1.1	−1.3± 2.1
Plasma S.G.: % change from initial level	−7.3± 2.8	−2.1± 2.4
Per cent of water dose excreted as total urinary water	45.3±13.7	92.1±22.6
Per cent of water dose excreted as water in excess of urinary isotonic sodium	22.3± 8.7	60.8±17.1

* All experiments were accompanied by a standard infusion of creatinine and PAH the volume of which has been included in the total water dose. Experiments on dog A during excessive DCA therapy have been excluded. The differences after cortisone are significant ($p < .01$).

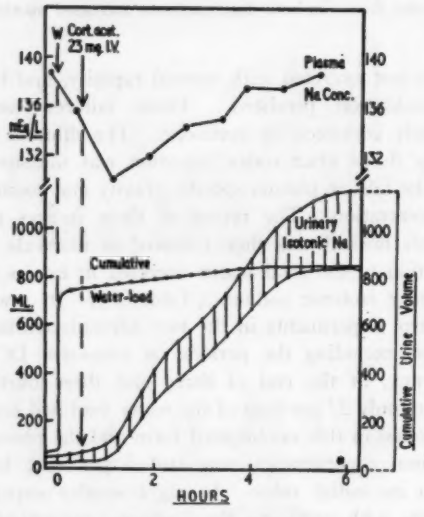

FIG. 10. THE EFFECT OF CORTISONE ON SODIUM AND WATER EXCRETION DURING DIURESIS, IN RELATION TO CHANGES IN PLASMA SODIUM CONCENTRATION (ADRENALECTOMIZED DOG A)

The dog was on optimal DCA therapy. The volumes of the priming and sustaining infusions of creatinine and PAH in distilled water have been included in the water load. The end of water diuresis coincided with the return of the plasma sodium concentration to its initial level.

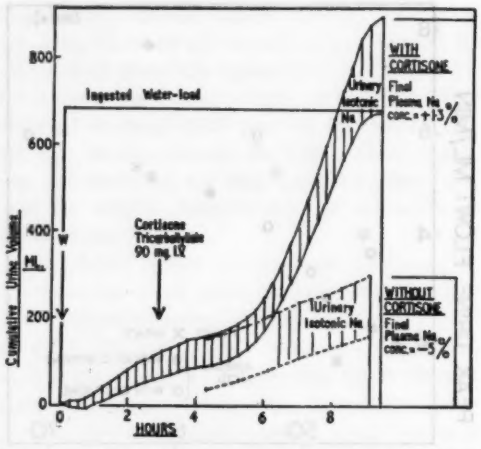

FIG. 11. WATER DIURESIS, WITH AND WITHOUT CORTISONE, IN ADRENALECTOMIZED DOG A

The two experiments were made without an infusion, when on optimal DCA therapy, with an interval of four days. At the end of diuresis after cortisone, the total water excreted in excess of total urinary isotonic sodium was equal to the water dose, and the plasma sodium concentration had returned to normal.

never exceeded it. By then the plasma sodium had returned to its initial concentration (Figures 10 and 11). The balance between the water load and the volume of unobligated water excreted was closest during cool weather, when insensible water loss was minimal. Thus, cortisone restored to normal the homeostatic function of water diuresis of correcting the plasma sodium hypotonicity, but did so at the expense of a negative balance of total body water.

DISCUSSION

Certain effects of adrenalectomy on the kidney are clearly secondary to loss of sodium. To measure other effects, which may not be dependent on sodium depletion, renal function must be studied under conditions of optimal bodily hydration, *i.e.*, in a state of sodium and water balance comparable to that existing before adrenalectomy. Such conditions are probably best obtained by combining daily injections of DCA with a constant diet and a small addition of sodium chloride. The advantages of such a regimen over one of DCA implantation are that the dose can easily be adjusted to meet any departure from the optimal

state, and that renal function can be assessed during its escape from DCA effect.

Using dogs of around 10 Kgm. weight, on an added salt intake of 2 gm. a day, other authors have shown that the minimal amounts of DCA needed for normal health and hydration are between 0.25 and 0.5 mg. a day (10, 11). With the same added salt, the dogs reported here needed 0.6 to 0.75 mg. a day which, allowing for their greater weight, is closely equivalent to the above amounts. Raising or lowering the dose of DCA by as little as 0.15 mg. was followed by consistent changes in body weight or in plasma specific gravity and electrolyte concentrations. Critical adjustment of the DCA therapy is thus essential for obtaining a steady optimal state, a precaution apparently overlooked in most previous studies.

After adrenalectomy the filtration rate seemed to vary with the state of bodily hydration, as was suggested by its inverse correlation with the plasma specific gravity, and by the over-retention of water which always accompanied the rise of filtration, whether chronic, as during excessive DCA therapy, or acute, from water ingestion or saline infusion. However, since filtration and renal blood flow remained subnormal even after optimal therapy had corrected any sodium depletion, at least a part of this failure could not be ascribed merely to reduction of the intravascular or extracellular fluid volumes. Some data on cardiac output are needed to throw further light on this problem.

The absence of any acute effect of DCA on filtration was in marked contrast with the rise in this function which occurred when excessive DCA therapy had caused over-retention of sodium and water. Though Davis and Howell found a subacute rise of filtration rate in the intact dog when DCA was given intramuscularly at more than twenty times the dosage used intravenously in the present experiments (32), this effect was considerably less than after cortisone or ACTH; and it is not clear from their data whether any sodium retention preceded the rise in filtration. It is possible, therefore, that DCA may have some primary action on the filtration rate when given in doses that are greatly excessive in terms of electrolyte effects.

The action of cortisone on the filtration rate and renal blood flow differs fundamentally from that of DCA in its rapidity of onset and its apparent independence of plasma volume or sodium balance. It would seem that cortisone has a "permissive" action of allowing a higher and more normal proportion to exist between the filtration rate and the level of bodily hydration. Whether this effect is confined to the kidneys or is part of a more widespread circulatory response cannot be decided from the present data.

The normal dog excretes a sodium load more rapidly than does man, and puts out only negligible amounts of sodium after 18 hours of fasting. The sodium output falls further during sustained maximal water diuresis (31), and, as has been shown in the present studies, either falls or increases only slightly when the filtration rate rises during acute water diuresis. After adrenalectomy, however, there is a partial loss of tubular ability to conserve sodium, and the filtration rate becomes the main regulator of sodium output. The adrenalectomized dog comes to resemble the salt-loaded normal dog in that both show a direct relation between sodium filtration and sodium output (33). This could be explained by assuming that salt-loading of the intact animal may suppress the secretion of a powerful sodium-retaining steroid, whereas in the normal fasting state this steroid [17] may already be present in amounts large enough to prevent any increase in sodium output when filtration rises after water ingestion.

Although its sodium-retaining action is well recognized (4), cortisone often increases sodium excretion (15, 18, 19, 36, 37). To explain this paradox, it has been suggested that under certain conditions, notably in the presence of DCA, cortisone may inhibit tubular reabsorption of sodium (15); and a concept has arisen of intratubular competition between cortisone and a steroid with more potent sodium-retaining effects, which cortisone is supposed to displace (17). This hypothesis ignores the important role of filtration rate in regulating sodium output. In the present studies it has been shown that after cortisone there is still a clear correlation between filtration rate and sodium output, though the latter is slightly less, and the fraction of filtered sodium reabsorbed slightly higher, than at parallel levels of filtration without cortisone. Thus, though cortisone in-

[17] Probably aldosterone (34, 35).

creases sodium reabsorption, this effect is not enough to prevent a net increase in sodium output in the presence of increased filtration of this ion. Similarly, Davis and Howell found in the intact dog that a great natriuresis accompanied the rise in filtration induced by cortisone or ACTH (32); and, though Roberts and Pitts did not find this effect in the adrenalectomized dog, it should be noted that there were no changes in filtration rate during the short periods of their experiments (14).

When DCA and cortisone were given together, reabsorption of sodium became almost complete, despite increased filtration. Similarly, Roberts and Pitts have shown that these two steroids have a summative effect on sodium reabsorption (14). Since the natriuretic effects of cortisone can now be adequately explained by increased filtration, there is no further need to invoke the hypothesis of competition between these steroids at the renal tubular level.

It is still a matter of dispute why adrenal failure should inhibit the capacity for water diuresis. The main issue concerns the cause of the renal failure to excrete a more hypotonic urine after water ingestion, despite adequate hemodilution (8, 13). Theoretically, this failure could be due either to extrarenal factors, such as continued secretion of antidiuretic hormone (ADH), or to intrinsic renal disturbances, conditioned by lack of adrenal hormones, and independent of the presence or absence of ADH. To consider first the present data on renal factors: when a normal filtration rate was restored by forced water ingestion during excessive DCA therapy, the ability to excrete a hypotonic urine, though still diminished, was greatly improved. Because the possibility of there having been any direct effect of DCA during these experiments seems to have been excluded by the high sodium outputs, and because DCA did not acutely correct filtration rate or water diuresis, it could be argued that the improved diuresis was somehow related to increased filtration. On the other hand, when cortisone restored the capacity for a normal water diuresis, the increased output of a more hypotonic urine sometimes occurred before filtration had risen. It is thus probable that an abnormality of tubular function, whether primary or secondary to ADH, is the essential factor in causing inhibition of water diuresis, though lowered filtration rate probably plays an additional role.

To consider next the evidence concerning extrarenal factors: it has been suggested that the action of ADH may be prolonged unduly in adrenal failure because of delayed inactivation of this hormone by the liver (38). However, this does not explain why water diuresis should end prematurely, after only a small fraction of the water load has been excreted, instead of continuing to increase slowly as the inactivation of ADH proceeds. If ADH be the paramount factor, it must therefore be postulated that this hormone is being continuously secreted during the long period of delayed diuresis. The secretion of ADH might so persist either because the osmoreceptors cannot function at their normal threshold when deprived of adrenal hormones, or because there is a further unidentified stimulus to ADH secretion, possibly humoral or arising from volume receptors.

SUMMARY

Changes in GFR, ERPF, electrolyte excretion, and water diuresis were studied in two adrenalectomized dogs in relation to bodily hydration and to acute administration of cortisone and DCA.

The dogs received daily DCA therapy which was assessed by its effects on bodily hydration as inadequate, optimal or excessive. Renal function was measured during the daily escape from DCA effect.

After adrenalectomy GFR and ERPF were subnormal on optimal DCA therapy, but rose after water-loading or saline infusion. DCA had no acute effect on GFR and raised it only when causing excessive retention of sodium and water. Cortisone raised GFR acutely, without change in plasma volume and despite a negative sodium balance.

After adrenalectomy, during escape from DCA effect, the sodium output varied directly with GFR. DCA caused acute maximal sodium retention. Cortisone usually increased the sodium output by raising GFR, thereby obscuring its other effect of slightly increasing tubular sodium reabsorption at any given level of filtration.

After adrenalectomy, water diuresis was greatly diminished, and was acutely restored by cortisone,

but not by DCA. This effect of cortisone was largely independent of GFR and electrolyte outputs.

After adrenalectomy, water ingestion caused prolonged hyponatremia which cortisone corrected by allowing excretion of the water load plus a volume of water isotonically equivalent to the urinary sodium. The plasma sodium concentration was thereby restored at the expense of total body water.

These results suggest that in the adrenalectomized dog: 1) There is a direct relationship between extracellular hydration and GFR, and between GFR and sodium output; and 2) that two adrenal steroids are needed to maintain normal renal function: (a) cortisone or hydrocortisone, to maintain normal GFR and water diuresis, and (b) a DCA-like steroid, to prevent excessive sodium excretion at normal or high levels of GFR.

Addendum

Since this paper was submitted for publication, acute effects of cortisone and DCA on GFR and sodium excretion in the adrenalectomized dog, which are similar to those reported here, have been described by Liddle, Pechet, and Bartter (39).

ACKNOWLEDGMENTS

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REFERENCES

1. Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J. L., Electrolyte balance studies in adrenalectomised dogs with particular reference to the excretion of sodium. J. Exper. Med., 1933, **57**, 775.
2. Harrop, G. A., Jr., Soffer, L. J., Ellsworth, R., and Trescher, J. H., Studies on the suprarenal cortex; III. Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in dog. J. Exper. Med., 1933, **58**, 17.
3. Harrison, H. E., and Darrow, D. C., Renal function in experimental adrenal insufficiency. Am. J. Physiol., 1939, **125**, 631.
4. Gaudino, M., and Levitt, M. F., Influence of the adrenal cortex on body water distribution and renal function. J. Clin. Invest., 1949, **28**, 1487.
5. Talbott, J. H., Pecora, L. J., Melville, R. S., and Consolazio, W. V., Renal function in patients with Addison's disease and in patients with adrenal insufficiency secondary to pituitary pan-hypofunction. J. Clin. Invest., 1942, **21**, 107.
6. Waterhouse, C., and Keutmann, E. H., Kidney function in adrenal insufficiency. J. Clin. Invest., 1948, **27**, 372.
7. Kottke, F. J., Code, C. F., and Wood, E. H., Urine dilution and concentration tests in adrenalectomised dogs. Am. J. Physiol., 1942, **136**, 229.
8. Roemmelt, J. C., Sartorius, O. W., and Pitts, R. F., Excretion and reabsorption of sodium and water in the adrenalectomised dog. Am. J. Physiol., 1949, **159**, 124.
9. White, H. L., Heinbecker, P., and Rolf, D., Further observations on the depression of renal function following hypophysectomy. Am. J. Physiol., 1949, **156**, 67.
10. Remington, J. W., Parkins, W. M., Swingle, W. W., and Drill, V. A., Efficacy of desoxycorticosterone acetate as replacement therapy in adrenalectomized dogs. Endocrinology, 1941, **29**, 740.
11. Cleghorn, R. A., Fowler, J. L. A., Wenzel, J. S., and Clarke, A. P. W., The desoxycorticosterone acetate requirement of the adrenalectomised dog. Endocrinology, 1941, **29**, 535.
12. Burnett, C. H., The actions of ACTH and cortisone on renal function in man *in* Renal Function, Transactions of the Second Conference, Oct. 1950, S. E. Bradley, ed., New York, Josiah Macy, Jr. Foundation, 1951, p. 106.
13. Burston, R. A., and Garrod, O., The variability of the lowered glomerular filtration rate in Addison's disease and panhypopituitarism and the effect of cortisone thereon. Clin. Sc., 1952, **11**, 129.
14. Roberts, K. E., and Pitts, R. F., The influence of cortisone on renal function and electrolyte excretion in the adrenalectomized dog. Endocrinology, 1952, **50**, 51.
15. Thorn, G. W., Forsham, P. H., Bennett, L. L., Roche, M., Reiss, R. S., Slessor, A., Flink, E. B., and Somerville, W., Clinical and metabolic changes in Addison's disease following the administration of compound E acetate. Tr. A. Am. Physicians, 1949, **62**, 233.
16. Garrod, O., and Burston, R. A., The diuretic response to ingested water in Addison's disease and panhypopituitarism and the effect of cortisone thereon. Clin. Sc., 1952, **11**, 113.
17. Adrenal Cortex: Transactions of the Third Conference, Nov. 1951, E. P. Ralli, ed., New York, Josiah Macy, Jr. Foundation, 1952.
18. Thorn, G. W., Engel, L. L., and Lewis, R. A., The effect of 17-hydroxycorticosterone and related adrenal cortical steroids on sodium and chloride excretion. Science, 1941, **94**, 348.

19. Davis, A. K., Bass, A. C., and Overman, R. R., Comparative effects of cortisone and DCA on ionic balance and fluid volume of normal and adrenalectomised dogs. Am. J. Physiol., 1951, **166**, 493.
20. Swingle, W. W., Collins, E., Barlow, G., and Fedor, E. J., Bioassay and physiological effects of cortisone on adrenalectomized dogs. Am. J. Physiol., 1952, **169**, 270.
21. Gregersen, M. I., and Stewart, J. D., Simultaneous determination of the plasma volume with T-1824, and the "available fluid" volume with thiocyanate. Am. J. Physiol., 1939, **125**, 142.
22. Berry, J. W., Chappel, D. G., and Barnes, R. B., An improved method of flame photometry. Indust. & Engin. Chem. (Anal. Ed.), 1946, **18**, 19.
23. Van Slyke, D. D., The determination of chlorides in blood and tissues. J. Biol. Chem., 1923, **58**, 523.
24. Folin, O., and Wu, H., A system of blood analysis. J. Biol. Chem., 1919, **38**, 81.
25. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. Clin. Invest., 1945, **24**, 388.
26. Fiske, C. H., and Subbarow, Y., The colorimetric determination of phosphorus. J. Biol. Chem., 1925, **66**, 375.
27. Gentskow, C. J., An accurate method for the determination of blood urea nitrogen by direct nesslerization. J. Biol. Chem., 1942, **143**, 531.
28. Phillips, R. A., Van Slyke, D. D., Dole, V. P., Emerson, K., Jr., Hamilton, P. B., and Archibald, R. M., Copper Sulphate Method for Measuring Specific Gravities of Whole Blood and Plasma. Glendale, Calif., Dan. Baxter, Inc., 1945.
29. Lowry, O. H., and Hunter, T. H., The determination of serum protein concentration with a gradient tube. J. Biol. Chem., 1945, **159**, 465.
30. Courtice, F. C., The blood volume of normal animals. J. Physiol., 1943, **102**, 290.
31. Gilman, A., Personal Communications.
32. Davis, J. O., and Howell, D. S., Comparative effect of ACTH, cortisone and DCA on renal function, electrolyte excretion and water exchange in normal dogs. Endocrinology, 1953, **52**, 245.
33. Thompson, D. D., and Pitts, R. F., Effects of alterations of renal arterial pressure on sodium and water excretion. Am. J. Physiol., 1952, **168**, 490.
34. Simpson, S. A., Tait, J. F., and Bush, I. E., Secretion of a salt-retaining hormone by the mammalian adrenal cortex. Lancet, 1952, **2**, 226.
35. Mach, R. S., Fabre, J., Duckert, A., Borth, R., and Ducommun, P., Action clinique et metabolique de l'Aldosterone (electrocortine). Schweiz. med. Wchnschr., 1954, **84**, 407.
36. Marcus, F., Romanoff, L. P., and Pincus, G., The electrolyte-excreting activity of adrenocortical substances. Endocrinology, 1952, **50**, 286.
37. Perera, G. A., Pines, K. L., Hamilton, H. B., and Vislocky, K., Clinical and metabolic study 11-dehydro-17-corticosterone acetate (Kendall compound E) in hypertension, Addison's disease and diabetes mellitus. Am. J. Med., 1949, **7**, 56.
38. Birnie, J. H., The inactivation of posterior pituitary antidiuretic hormone by liver extracts. Endocrinology, 1953, **52**, 33.
39. Liddle, G. W., Pechet, M. M., and Bartter, F. C., Enhancement of biological activities of corticosteroids by substitution of halogen atoms in 9 α position. Science, 1954, **120**, 496.

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THE EFFECT OF THE SUPINE POSITION ON RENAL FUNCTION IN THE NEAR-TERM PREGNANT WOMAN [1, 2]

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(Submitted for publication December 13, 1954; accepted February 2, 1955)

It has been demonstrated previously that urine flow in the recumbent near-term pregnant woman is appreciably less when she is lying on her back than when she is lying on her side and that without change of position the relative oliguria persists for several hours before it is overcome (1, 2).

The present report extends the observations on the effect of position on urine flow to include studies of sodium and potassium excretion, effective renal plasma flow, and glomerular filtration rate in near-term normal pregnant women, in women with eclamptogenic toxemia of pregnancy, and in women with large abdominal neoplasms originating in the pelvis. Attempts have been made to determine the mechanism by which the various observed changes in renal function are produced.

METHODS

Studies were made 11 times in 10 near-term normally pregnant women and 13 times in 8 women with preeclampsia. In most of these studies the excretion of sodium and potassium, the effective renal plasma flow (RPF) and the glomerular filtration rate (GFR) were measured. In one normal and one preeclamptic pregnant woman the observations were repeated following the insertion of large-bore heavy-walled ureteral catheters into both ureters well above the level of the pelvic brim. Similar renal function studies were made in two women who had large abdominal neoplasms arising from the pelvis, one an ovarian serous cystadenocarcinoma and the other a uterus containing multiple fibromyomas. Both tumors were approximately the size of a uterus at 32 to 34 weeks' gestation. Seven patients who had either delivered 7 or 8 days previously or who had undergone total hysterectomy 9 or 10 days before were also observed. Three subjects of this group had been studied previously while pregnant.

All medications were stopped the night before; 2 hours before starting the study, a breakfast of milk, cereal, and toast was allowed. One-half hour later an indwelling catheter was inserted into the bladder and oral hydration consisting of 100 ml. of water every 20 minutes was initiated. At this time in the 17 studies where effective renal plasma flow and inulin clearances were to be measured sodium paraminohippurate and inulin were given intravenously in suitable priming doses, followed by a sustaining infusion of each administered intravenously at a constant rate of 4 to 5 ml. per minute. Urine was collected in periods averaging 20 minutes each. With each collection the bladder was washed with distilled water followed by air. In 14 of the studies only the clearances of endogenous creatinine were determined.

Paraminohippurate was measured by the method of Bratton and Marshall (3), inulin by the method of Higashi and Peters (4), and creatinine by the method of Bonsnes and Taussky (5). Sodium and potassium were determined with the Beckman flame photometer.

Usually the initial observations were made in the lateral recumbent position. One hour after starting oral hydration and one-half hour before starting the first collection period the patient was limited to lying on her side. Two to three collections were made while she was lying in the lateral recumbent position and then the subject was turned on her back, the head being maintained at the same level. After three to six collection periods on her back she was returned to her side for two or three periods. In a few studies the opposite approach was used, starting with the patient on her back, then turning her on her side, and finally returning to her back.

In studying two normal near-term pregnant and two normal post-hysterectomy subjects blood pressure cuffs were applied loosely high on each thigh prior to study. Initially, two to three observations were made with the patient in the supine position and then she was turned on her side. After three to four periods on the side the blood pressure cuffs were inflated and their pressures maintained at 40 to 60 mm. Hg. After three to four periods the pressure was released and with the patient still on her side two or three more collections were made.

Brachial arterial blood pressures determined with a cuff and the radial pulse rates were measured at the time of administration of water and at the end of each urine collection period. Care was taken to maintain the comfort of the patient and to minimize apprehension.

OBSERVATIONS

Urine flow in the lateral recumbent position ranged from 4 to 12 ml. per minute. In this posi-

[1] This study was assisted by a grant from the Cleveland Area Heart Society.

[2] Presented in part at the Fortieth Annual Clinical Congress of the American College of Surgeons, Atlantic City, N. J., November 16, 1954.

TABLE I

Near-term non-toxemic pregnant women—The per cent change in renal function when supine compared to lying on the side

Patient	Urine flow % Change	Na/Min. % Change	K/Min. % Change	RPF % Change	GFR % Change
H. J.	−31	−27	−18	+ 8	+ 1
L. M.	−63	−68	−22	—	−14*
S. M.	−18	−35	− 6	− 4	+ 3
M. B.	−45	−36	− 4	− 8	− 6
J. W.	−20	−19	0	—	− 2*
P. M.	−37	−44	−18	—	− 8*
N. T.†	−62	−54	—	—	—
N. S.	−54	−45	—	—	—
A. M.	−36	−50	−40	—	− 6*
B. J. ‡(1)	−68	−62	−21	−28	−23
(2)§	−64	−60	−16	−24	−18
Average	−43%	−44%	−16%	−7.5%	−6.5%

* Endogenous creatinine clearance.
† Twins.
‡ Thirty-six weeks' pregnant plus large uterine fibromyomas.
§ Bilateral ureteral catheters inserted beyond brim of pelvis.

tion the excretion of sodium and potassium per minute ranged from 58 to 240 microequivalents and 46 to 198 microequivalents, respectively, while RPF and GFR ranged from 325 to 740 ml. and 84 to 165 ml., respectively. The lower values usually occurred in the preeclamptic group. The blood pressure and pulse rate were not significantly different in the two positions.

TABLE II

Near-term preeclamptic pregnant women—The per cent change in renal function when supine compared to lying on the side

Patient	Urine flow % Change	Na/Min. % Change	K/Min. % Change	RPF % Change	GFR % Change
D. J.	−31	−31	−15	−14	− 9
L. B.*(1)	−48	−40	−32	−22	−23
(2)	+ 5	+ 2	+ 5	+ 4	− 2
(3)	−36	−30	—	−18	−16
J. V. (1)	−73	−70	—	− 4	− 3
(2)	− 6	−10	−12	+ 2	− 3
K. M.	−66	−56	−28	—	−12†
V. P.	−31	−29	− 8	—	+ 3†
E. H.	−44	−34	−18	−14	− 8
K. S.*‡(1)	−81	−63	—	−39	−33
(2)	−69	−48	+ 2	−36	−30
(3)§	−63	−67	− 9	−34	−27
M. W.	−43	−40	−28	—	−18†
Average	−44%	−39%	−15%	−15%	−11%

* About one week intervals.
† Endogenous creatinine clearance.
‡ Twins.
§ Bilateral ureteral catheters inserted beyond brim of pelvis.

In 22 of the 24 studies on 18 near-term pregnant women the supine position compared to the lateral recumbent was associated with moderate to marked and nearly parallel reductions in the rate of urine flow and sodium excretion (Table I, II). The excretion of potassium, in general, was moderately decreased but the changes were much more variable than for sodium and water. Little difference was found in the effect of the supine position on RPF, GFR, sodium excretion, or urine flow between the normal and toxemic pregnant groups. The responses of RPF and GFR to the supine position showed considerable variation from patient to patient. Three patterns were noted. In some there was an appreciable decrease in RPF and GFR which was maintained throughout the supine periods. A typical example is shown in Figure 1. In others there was an initial drop in RPF and GFR followed by recovery even though the supine position was maintained and sodium and water excretion continued to be depressed. A third group, while developing significant decreases in sodium and water excretion during the supine periods, showed no real change in RPF or GFR.

The decreases in water and sodium excretion while lying on the back were usually not immedi-

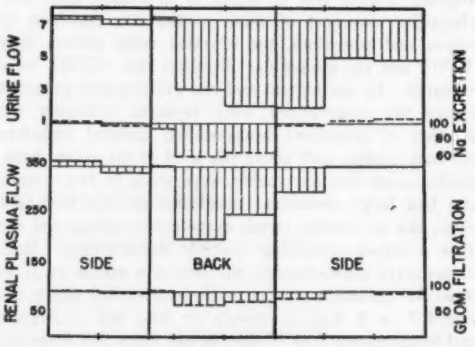

FIG. 1. THE EFFECT OF THE SUPINE POSITION ON URINE FLOW, SODIUM EXCRETION, EFFECTIVE RENAL PLASMA FLOW AND GLOMERULAR FILTRATION RATE IN THE NEAR-TERM SUBJECT K. S. WITH PREECLAMPSIA AND TWINS

Urine flow, renal plasma flow, and glomerular filtration rate are expressed as ml. per Min.; sodium excretion is expressed as microequivalents per minute. The total time in minutes for each position, side, back, and again side, were 42, 55, and 63 minutes, respectively.

TABLE III

Non-pregnant women with large abdominal neoplasms—The per cent change in renal function when supine compared to lying on the side

Patient	Urine flow % Change	Na/Min. % Change	K/Min. % Change	GFR* % Change
H. S.†	−41	−34	−11	−14
B. J.‡	−66	−51	−25	−20
Average	−53%	−42%	−18%	−17%

* Endogenous creatinine clearance.
† Ovarian serous cystadenocarcinoma.
‡ Large uterine fibromyomas.

ately maximal but became so only after 10 to 20 minutes in the supine position; when the patient again was placed on her side, recovery frequently was similarly delayed. Figure 1 demonstrates a typical response.

In the two patients in whom no reduction in RPF, GFR, sodium excretion, or urine flow was noted while supine, the studies were carried out more than once. One (L. B., Table II) on two other occasions, one week before and one week after, showed significant decreases in the supine position. The other (J. V., Table II), when placed in the supine position, demonstrated a marked decrease in water and sodium excretion the first time studied but 24 hours later failed to show a similar response.

Positional alteration from side to back in the two women with large abdominal neoplasms arising from the pelvis (Table III) produced changes in urine flow, sodium and potassium excretion and GFR as estimated by the clearance of endogenous creatinine comparable to those seen in the near-term pregnant subjects. In the seven patients who either had been delivered or who had undergone hysterectomy 7 to 10 days before study very little change was noted in these components of renal function when the recumbent subjects were turned from the side to the back (Table IV).

TABLE IV

Postpartum or post-hysterectomy women—The per cent change in renal function when supine compared to lying on the side

Patient	Urine flow % Change	Na/Min. % Change	K/Min. % Change	RPF % Change	GFR % Change
K. S.*	+ 8	+ 5	− 2	+3	−2
D. J.*	+ 2	+ 5	−13	+1	+4
J. V.†	−30	−10	− 6	—	−3‡
H. S.†	− 4	+ 3	+ 7	—	−3‡
P. T.†	−10	−14	+ 3	—	−8‡
L. R.†	− 2	− 5	− 3	—	−4‡
J. W.*	−12	− 8	+ 1	—	−3‡
Average	− 7%	− 3%	− 2%	+2%	−3%

* Seven or more days postpartum.
† Nine or more days post-hysterectomy.
‡ Endogenous creatinine clearance.

Two subjects (B. J., Table I, and K. S., Table II) showed functional alterations in the supine position almost identical to those previously observed in each, although ureteral compression by the pregnant uterus had been made virtually impossible by the presence of large-bore heavy-walled ureteral catheters inserted bilaterally into the ureters well beyond the level of the brim of the pelvis.

Sodium and water excretion while supine was compared to the excretion during femoral venous compression in the lateral recumbent position. Four women, two near-term pregnant and two non-pregnant, were studied. In the recumbent pregnant group the shift from the back to the side resulted in an appreciable increase in urine flow and sodium excretion but in the non-pregnant subjects the same change of position produced no change. While remaining in the lateral recumbent position, blood pressure cuffs high on each thigh were inflated to subdiastolic pressures of

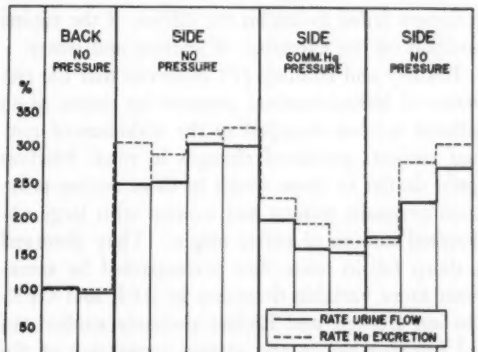

FIG. 2. THE PERCENTAGE CHANGE IN SODIUM AND WATER EXCRETION IN A NEAR-TERM PREGNANT SUBJECT L. M. WHEN TURNED FROM LYING ON HER BACK TO LYING ON HER SIDE AND THEN WITH AND WITHOUT BLOOD PRESSURE CUFFS HIGH ON EACH THIGH INFLATED TO 60 MM. HG

The total time in each of the four procedures was 39, 74, 55, and 56 minutes. Urine flow and sodium excretion while on the back averaged 1.53 ml. and 43 microequivalents per minute.

40 to 60 mm. Hg. In all women, pregnant and non-pregnant, this was followed by a significant fall in sodium and water excretion. Release of the cuff pressure resulted in recovery. The observed changes in one near-term pregnant woman are shown in Figure 2.

DISCUSSION

There are many possibilities regarding the genesis of these marked reductions in urine flow and sodium excretion observed in the supine subject with a large pregnant uterus or with a large abdominal tumor arising from the pelvis. Among them are compression of the iliac veins at the pelvic brim, compression of the inferior vena cava either below or above the level of the renal veins or both, compression of the extrarenal portions of the renal veins, or compression of the kidney itself. Not encountered in any of these subjects and therefore excluded as a cause was the so-called supine hypotensive syndrome, in which merely positioning a woman late in pregnancy on her back results in arterial hypotension (6). Any ureteral compression at the brim of the pelvis produced by the large pregnant uterus did not appear to cause the reductions in sodium and water excretion in the supine position since in two cases the presence of large-bore heavy-walled ureteral catheters failed to inhibit the effects of the supine position on the excretion of sodium and water.

Bradley and Bradley (7) observed that the elevation of intraabdominal pressure by means of an inflated balloon strapped to the abdomen of normal subjects produced changes in renal function quite similar to those found in these supine near-term pregnant women and women with large abdominal tumors of pelvic origin. They observed a sharp fall in urine flow accompanied by somewhat more variable decreases in RPF and GFR. Farber, Becker, and Eichna similarly studied renal function before and during congestion of the inferior vena cava using a venous catheter with an attached inflatable balloon (8). Following inflation of the balloon there were changes in renal function similar to those noted by Bradley and Bradley and those observed in the supine women. The degree of change was less marked if the venal caval congestion was produced below the level of the renal veins than if it was created above that level. Other investigators, by applying tourniquets to the thighs at subdiastolic pressures to cause femoral venous compression, produced comparable changes in the excretion of sodium and water (9, 10).

Femoral venous pressures have been observed in non-pregnant subjects to be only slightly increased in the supine position whereas in term pregnant individuals in the supine position they are almost twice as great as in the lateral recumbent position (6). The similarities in the changes in renal function noted in the present experiments in supine subjects with either large pregnant uteri or with large pelvic tumors to the changes produced by the compression of either the inferior vena cava or the femoral veins suggest that the decreases in sodium and water excretion in the supine position may also result from comparable venous compression.

The extent of the venous pressure elevation above the femoral veins in supine pregnant women does not appear to have been investigated. Whether this position results in venous pressure elevations only in the tributaries distal to the inferior vena cava at and below the level of the brim of the pelvis or whether there is also augmentation of venous pressure in the vena cava and renal veins when supine is not known. Studies in progress include the measurement of pressures in the supine position at various levels of the inferior vena cava by means of venous catheters. It is hoped that by such studies it will be possible to account for the occasional absence of reduction in water and sodium excretion in near-term pregnant women in the supine position, for it is conceivable that the degree of venous compression by the uterus and its contents may vary not only from subject to subject but also may vary in the same subject at different times depending on the anatomical relationship of the fetus and uterus to the venous return system.

SUMMARY

Shifting near-term pregnant women from lying on their sides to lying on their backs resulted in a considerable decrease in the rate of excretion of water and sodium. The excretion of potassium and effective renal plasma flow and glomerular filtration rates usually were not as markedly affected.

The renal response to the supine position was not peculiar to pregnancy for similar changes were observed in women with large abdominal neoplasms pelvic in origin.

The mechanism by which these changes were produced remains somewhat obscure but appears to involve venous compression rather than ureteral obstruction.

ACKNOWLEDGMENTS

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REFERENCES

1. Walker, E. W., McManus, M., and Janney, J. C., Kidney function in pregnancy. II. Effect of posture on diuresis. Proc. Soc. Exper. Biol. & Med., 1933–34, **31**, 392.
2. Hendricks, C. H., and Barnes, A. C., The effect of the supine position on urinary output in pregnancy. Am. J. Obst. & Gynec. In press.
3. Bratton, A. C., and Marshall, E. K., Jr., A new coupling component for sulfanilamide determination. J. Biol. Chem., 1939, **128**, 537.
4. Higashi, A., and Peters, L., A rapid colorimetric method for the determination of inulin in plasma and urine. J. Lab. & Clin. Med., 1950, **35**, 475.
5. Bonsnes, R. W., and Taussky, H. H., On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 1945, **158**, 581.
6. Howard, B. K., Goodson, J. H., and Mengert, W. F., Supine hypotensive syndrome in late pregnancy. Obst. & Gynec., 1953, **1**, 371.
7. Bradley, S. E., and Bradley, G. P., The effect of increased intra-abdominal pressure on renal function in man. J. Clin. Invest., 1947, **26**, 1010.
8. Farber, S. J., Becker, W. H., and Eichna, L. W., Electrolyte and water excretions and renal hemodynamics during induced congestion of the superior and inferior vena cava of man. J. Clin. Invest., 1953, **32**, 1145.
9. Fitzhugh, F. W., McWhorter, R. L., Estes, E. H., Jr., Warren, J. V., and Merrill, A. J., The effect of application of tourniquets to the legs on cardiac output and renal function in normal human subjects. J. Clin. Invest., 1953, **32**, 1163.
10. Wilkins, R. W., Tinsley, C. M., Culbertson, J. W., Burrows, B. A., Judson, W. E., and Burnett, C. H., The effects of venous congestion of the limbs upon renal clearances and the excretion of water and salt. I. Studies in normal subjects and in hypertensive patients before and after splanchnicectomy. J. Clin. Invest., 1953, **32**, 1101.

EFFECT OF ACUTE CLINICAL STRESS ON THE LEVELS OF HEXOSAMINE IN SERUM AND ITS EXCRETION IN URINE

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The total serum hexosamine [2] level is elevated in many unrelated chronic diseases (1, 2), and in experimental animals following acute injury (3, 4). It is not clear whether this elevation is due to increased production, diminished utilization or merely a shift of tissue hexosamines into the circulation. Since the activity of the adrenal glands is increased in many of these conditions of acute and chronic stress, the role of the adrenal cortex in controlling serum hexosamine levels has been studied. During the course of these studies, it was noted that normal urine contained relatively large amounts of hexosamine, which also increased during conditions of stress. The excretion of hexosamine in the urine in a variety of clinical situations has been measured, therefore, for the purpose of defining factors which alter the rate of its excretion and of correlating excretion levels with changes in serum hexosamine.

An ion exchange technique has been applied to urine hydrolysates for the purpose of separating hexosamines from the many chromogens in urine which interfere with the colorimetric determination of hexosamine (5). Previously reported studies on urinary hexosamine have not taken this into account and consequently report erroneously high values (6, 7).

METHODS

All patients selected for urinary hexosamine determinations were free of demonstrable renal disease as indicated by their clinical histories, by blood urea nitrogen determinations, and by routine examination of the urine for cells, casts, and protein. All urines were refrigerated (4° C.) without preservatives until the completion of each collection, and then frozen until ready for analysis.

For the determination of hexosamine an aliquot of urine (1 ml.) or plasma (0.1 ml.) was hydrolyzed in 2N hydrochloric acid for 15 hours at 100° C. After dilution and filtration, the hexosamine was isolated from the hydrolysate on Dowex-50 and determined by a modification of the method of Elson and Morgan (5). All urine samples and the serum from the case of gout were analyzed by the above method. The remaining serum hexosamine determinations were carried out by a previous modification of the method of Elson and Morgan (2). In this report, serum hexosamine values are expressed as mg. per cent change from an observed control value in the same individual. Where control values were unattainable such as in the cases of myocardial infarction, the hexosamine levels are expressed as milligrams per cent change from the mean hexosamine level observed in a series of normal individuals (107 mg. per cent) (2).

Electrophoresis of serum proteins was carried out on Whatman No. 1 filter paper sheets using a horizontal apparatus (Arthus H. Thomas, Co.). For the separation of proteins, 0.01 to 0.02 ml. of plasma were applied on a 25 mm. line. For hexosamine studies 0.1 ml. was applied to a 150 ml. line. Separations were carried out in pH 8.6 veronal buffer (ionic strength 0.06) at room temperature and constant current (10mA) for five to six hours. The voltage generally fell from 250 to 190 V during the separation. At the completion of a separation the paper was dried in an oven at 110° C. for 20 minutes, stained for 7 minutes in an alcoholic solution of 0.1 per cent bromphenol blue saturated with mercuric chloride (8), rinsed in tap water and dried again in an oven at 110° C. For an analysis of the protein components, optical density of a dyed strip was read at 600 mμ in a Beckman Spectrophotometer (Model DU) by the use of a specially constructed strip holder. A protein distribution curve was obtained by charting optical density (ordinate) against distance migrated (abscissa) on graph paper. The protein distribution was measured by cutting the charted electrophoretic curve into its various protein components, weighing each component, and relating the weight to the total weight of all components.[3] The elec-

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[2] The naturally occurring hexosamines are glucosamine and galactosamine. The methods used here do not distinguish between these two amino sugars.

[3] No corrections were made for the variation in dye-binding capacity of the different proteins (9).

TABLE I

Constancy of excretion of hexosamine in the urine

Diagnosis	No. obs.	Observation	Mean	V*	Range
Scleroderma (27 yr. ♀)	74	24 hr. urine volume (*ml.*)	1,480	±18	750–2,000
	31	Hexosamine (*mg./24 hr.*)	76.5	± 9	65–88
	63	Creatinine (*mg./24 hr.*)	841	±10	558–1,007
Ankylosing spondylitis (40 yr. ♀)	7	24 hr. urine volume (*ml.*)	1,600	±34	740–2,050
	7	Hexosamine (*mg./24 hr.*)	91.7	± 5	85–99
Myxedema (54 yr. ♀)	60	24 hr. urine volume (*ml.*)	1,940	±15	1,000–2,340
	28	Hexosamine (*mg./24 hr.*)	51.4	± 9	42–60

* V = Coefficient of Variation = $\frac{100\sqrt{\frac{\Sigma(x-\bar{x})^2}{N-1}}}{M}$.

trophoretic distribution of hexosamines was determined by cutting the paper according to the known protein bands. Each band of paper was then hydrolyzed in 3 ml. of 1N hydrochloric acid for 15 hours and after isolation on Dowex-50, the hexosamine contents were determined (5).

RESULTS

Constancy of hexosamine excretion

Six normal adult men and four women were found to excrete approximately the same daily amounts of hexosamine in the urine (84 mg. per 24 hours), with moderate variations (± 12 mg. per 24 hours) from person to person. Values above 100 and below 65 mg. per 24 hours were beyond the observed normal range. The daily rate of excretion, however, in the same subject was more constant (Table I). One patient with scleroderma was studied for a period of three months during which time urine volume, hexosamine and creatinine determinations were made. Using the coefficient of variation (V) to compare the variability of these different observations, it can be seen that the excretion of hexosamine was as constant as the excretion of creatinine, despite wide variations in urine volume. A low degree of variability in the excretion of hexosamine was also observed in a patient with rheumatoid arthritis studied for seven consecutive days and in a patient with myxedema studied for two months. There was no apparent influence of the menstrual cycle on the excretion of hexosamine in the urine of two women with scleroderma who had normal cycles and whose daily urine collections had been analyzed for three months.

The hourly excretion rate of hexosamine in the urine was studied in one patient with scleroderma and found to be fairly constant, despite wide variations in the rate of urine formation (Table II). A reduction in hexosamine excretion during the hours from 8:00 P.M. to midnight was observed on two consecutive days.

Urine hexosamine in chronic diseases

Urinary hexosamine excretion was first studied in normal individuals and later in patients with a variety of chronic diseases in order to assess normal and abnormal excretion limits (Table III). All of the urine specimens represented 24-hour collections during periods when no medication was being administered, with the exception of a few patients with rheumatoid arthritis who were being

TABLE II

Rate of excretion of hexosamine in urine of patient with scleroderma

Day	Collection period	Urine volume (*ml./hr.*)	Hexosamine (*mg./hr.*)
1	8 A.M.–12 N.	144	3.5
	12 N.–4 P.M.	80	3.5
	4 P.M.–8 P.M.	84	3.5
	8 P.M.–12 M.	12	1.2
	12 M.–8 A.M.	39	2.5
2	8 A.M.–12 N.	139	3.2
	12 N.–4 P.M.	59	4.0
	4 P.M.–8 P.M.	54	3.8
	8 P.M.–12 M.	38	2.5
	12 M.–8 A.M.	72	3.9
3	8 A.M.–8 A.M.	95	3.5
4	8 A.M.–8 A.M.	77	3.0
5	8 A.M.–8 A.M.	24	3.4
6	8 A.M.–8 A.M.	36	3.7

TABLE III

Levels of urinary excretion of hexosamine in a variety of clinical diseases

Diagnosis	No.	Hexosamine (*mg./24 hrs.*)
Metastatic carcinoma of colon	1	76
Scleroderma	3	82±5*
Rheumatoid arthritis	9	94±15
Islet cell tumor of pancreas	1	104
Portal cirrhosis	1	112
Metastatic carcinoma of rectum	1	123
Metastatic carcinoma of breast	1	126
Pancreatitis with obstructive jaundice	1	137
Acute hepatitis with jaundice	1	142
Normal adults	10	84±12

* Standard deviation.

treated with salicylates. The excretion of hexosamine was increased in many instances without any apparent relationship to the specific nature of the illness. In some illnesses it was relatively unaffected. Only two of nine patients with active rheumatoid arthritis excreted more than 100 mg. per 24 hours.

Serum and urine hexosamine in acute clinical stress

In order to study some of the physiological factors related to the increased urinary excretion and elevated serum hexosamine levels observed in chronic diseases, cases of acute spontaneous and induced clinical stress were studied. Some of the responses of serum hexosamine levels to acute stress are shown in Figure 1.

Myocardial infarction: Two cases of acute myocardial infarction each demonstrated an abrupt increase in the serum hexosamine levels from assumed normal levels, which gradually returned toward normal again after one to two weeks.

Epinephrine administration: In an attempt to induce a stress response, two normal men were given large doses of epinephrine, each receiving 2.2 mg. subcutaneously, daily in divided doses for five days. Despite evidence of epinephrine effect (nervousness, tachycardia, *etc.*) the serum hexosamine levels were not significantly altered.

Surgical operations: The serum hexosamine levels were studied in three normal adult female patients who were operated upon for gynecological conditions: A. T. had a multiple myomectomy for uterine fibroids, P. W. had an hysterectomy and right salpingo-oophorectomy for uterine fibroids, and S. M. had an ovariectomy for an ovarian cyst. All developed sharp increases in serum hexosamine levels, reaching their peaks at approximately one week after operation.

The excretion of urine hexosamine was also studied in cases of acute surgically-induced stress

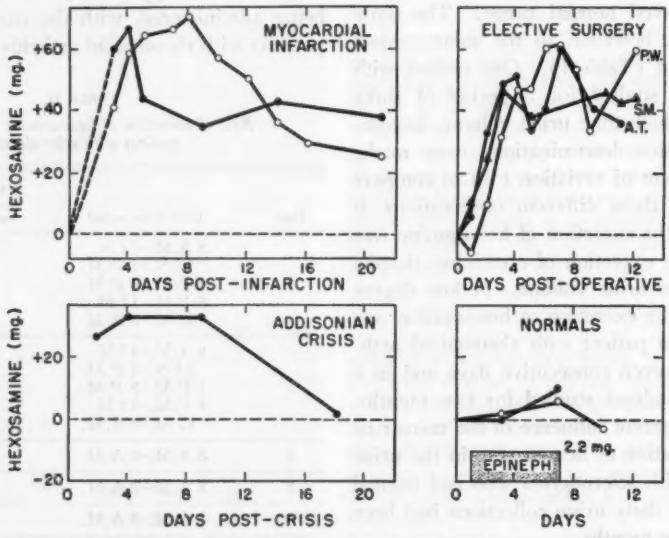

FIG. 1. EFFECT OF ACUTE CLINICAL DISEASES, OPERATIONS, AND TREATMENT WITH EPINEPHRINE ON THE LEVELS OF HEXOSAMINE IN THE SERUM

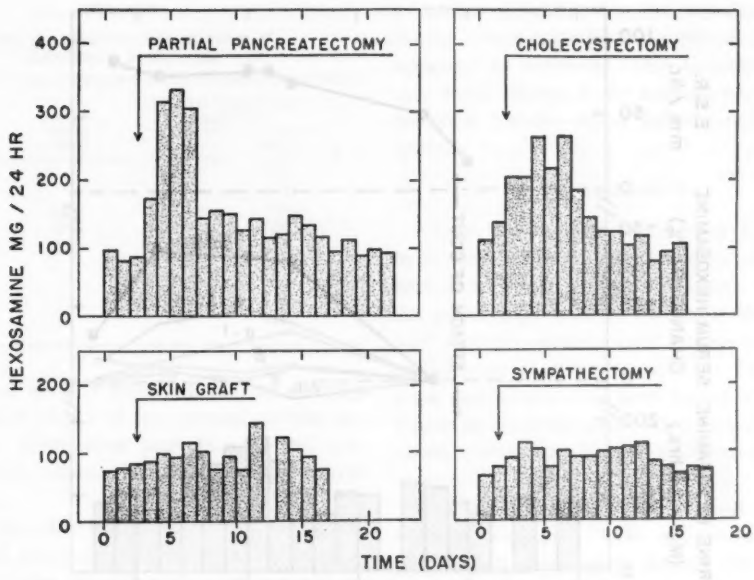

FIG. 2. EFFECT OF SURGICAL OPERATIONS ON THE EXCRETION OF HEXOSAMINE IN THE URINE

(Figure 2). The patients consisted of a 25-year old man who had surgical removal of an islet cell tumor of the pancreas, a 55-year old man with obstructive jaundice due to pancreatitis on whom a cholecystectomy and drainage of the common bile duct were performed, a 43-year old woman who had a skin graft on the site of a previous radical mastectomy scar, and a 24-year old woman with scleroderma who had a lumbodorsal sympathectomy. All demonstrated increased excretion of urine hexosamine exceeding the observed normal range, rising in one case to 300 mg. per 24 hours (pancreatectomy), and returning to normal in two to three weeks in all patients.

Acute gouty arthritis: A 46-year old man, while under observation for another study, developed a spontaneous attack of gout with marked involvement of the big toe, ankle and knee of the same leg (Figure 3). This attack was followed by an increase in serum hexosamine which occurred simultaneously with increased excretion of urinary hexosamine and an acceleration of the blood sedimentation rate. Analysis of the electrophoretically separated serum proteins demonstrated that the increase in serum hexosamine was almost entirely confined to the hexosamine in the alpha globulin fractions. No statistically significant changes in the relative amounts of the serum protein fractions were detectable. The attack of gout subsided after 10 days and was followed by a return toward normal of the plasma and urine hexosamine.

Influence of adrenal cortex on serum and urine hexosamine

Since the adrenal glands become physiologically hyperactive following acute injury or stress, and since serum levels and urinary excretion of hexosamine increased following stress, the role of the adrenal cortex as a mediator in this response was considered.

To study serum hexosamine changes in the absence of normal adrenocortical function, a 17-year old boy with known Addison's disease was observed. He entered the hospital in acute Addisonian crisis with a temperature of 104°, due to infectious mononucleosis. His serum hexosamine levels had been normal prior to this admission but became transiently elevated with this acute illness (Figure 1). The patient died several weeks later

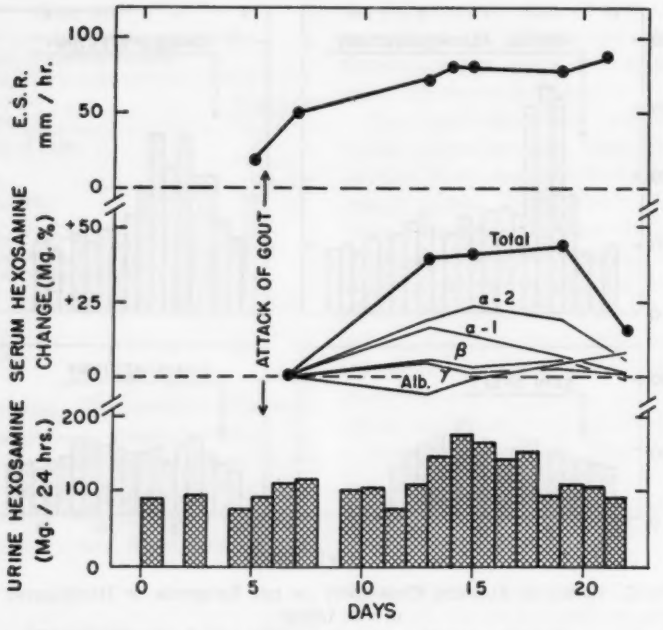

FIG. 3. EFFECT OF AN ACUTE ATTACK OF GOUTY ARTHRITIS ON THE DISTRIBUTION OF HEXOSAMINE IN THE SERUM, AND THE EXCRETION OF HEXOSAMINE IN THE URINE

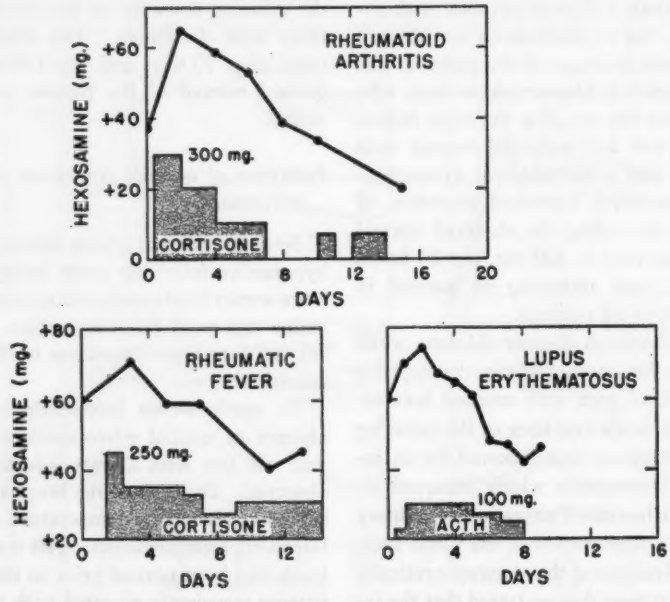

FIG. 4. RESPONSE OF ELEVATED SERUM HEXOSAMINE LEVELS TO TREATMENT WITH CORTICOTROPIN (ACTH) AND CORTISONE

TABLE IV

Effect of corticotropin on excretion of hexosamine in the urine

Day	Corticotropin (*units*)	Urine hexosamine (*mg./24 hrs.*) L. B.	N. K.
1		82	152
2		90	152
3	100	76	135
4	100	96	136
5	100	72	141
6		77	121
7		98	122
8		86	131
9		90	125

as a result of another infection, and at autopsy, there was no evidence of any adrenal cortical tissue present. Four other patients with well-controlled Addison's disease had normal serum hexosamine levels.

To study the effect of adrenal cortical hormones, patients with abnormally elevated serum hexosamine levels were treated with corticotropin or cortisone. Examples in patients with rheumatic fever, rheumatoid arthritis, and lupus erythematosus are shown in Figure 4. In each instance, hormonal treatment resulted in a transient increase in hexosamine levels followed by a return toward normal, while the hormone was still being given. This is contrary to what one would expect if the adrenal cortex had produced an hexosamine-increasing factor. One normal individual and four patients with hyperthyroidism who had normal hexosamine levels before treatment received corticotropin (100 mg.[4] per day for 5 to 10 days) and no changes in hexosamine levels resulted.

The effect of ACTH on the urinary excretion of hexosamine was also studied (Table IV) in two patients each of whom received 100 units of corticotropin daily intramuscularly in divided doses for three days. Patient L. B. was a 45-year old woman with inactive rheumatoid arthritis who was excreting normal amounts of hexosamine. This excretion rate was uninfluenced by corticotropin. The other patient, N. K., a 16-year old boy who was recovering from a leg operation, had an increased rate of excretion of hexosamine before treatment with corticotropin. The urinary excretion of hexosamine did not increase further during treatment with corticotropin, but actually appeared to decrease. This is contrary to what one would expect if the adrenal cortex were responsible for the stress induced hexosamine response.

[4] Since these experiments were conducted prior to the unit standardization of ACTH, the dosage is expressed in milligrams.

DISCUSSION

Serum hexosamine levels have been reported to be elevated in many unrelated chronic diseases such as leukemia, Hodgkin's disease, carcinomatosis, periarteritis nodosa, rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus and chronic infections (1, 2, 10). Serum hexosamine levels have also been found to be elevated following acute diseases such as pneumonia, myocardial infarction (1) and experimental injury to animals (3, 4, 11).

The data presented here confirm the observations of West and Clarke (1) on myocardial infarction but demonstrate further that other types of unrelated acute diseases and injuries to the body can also induce an increase in serum hexosamine. In one instance (case of gout) the increase in serum hexosamine was found to be in the hexosamines contained in the alpha-globulin fractions. This is consistent with observations that the alpha-globulins (12) and hexosamine-rich alpha-globulin mucoproteins (13, 14) increase in response to other acute injuries or diseases such as burns (12) and pneumonia (13, 14).

Glucosamine has been identified as an important component of urinary mucus (15, 16), a carbohydrate-rich material long recognized as a normal constituent of urine (15, 17). A chemical characterization of most of the glucosamine-containing substances in the urine, however, has not been made. It has recently been shown that approximately 40 per cent of the total urine hexosamine is contained in low molecular weight freely dialyzable substances, and that almost all of the non-dialyzable urine hexosamine was found to migrate electrophoretically at pH 8.6 as a single mucoprotein peak (18) which may be similar in part to the urine mucoprotein isolated by Tamm and Horsfall (19).

It was observed that following acute diseases or body injury, the increased excretion of urine hexosamine coincided in time with the increase seen in serum hexosamine levels. Until the hexosa-

mine-containing substances in serum and urine are more clearly identified, however, their physiological relationship cannot be established.

The increase in serum hexosamine levels and in the urinary excretion of hexosamine following acute diseases or injury does not appear to be mediated by the adrenal cortex for the following reasons: 1) A patient with Addison's disease developed a typical increase in serum hexosamine during a crisis, 2) corticotropin or cortisone administration was followed by a reduction in the abnormally elevated hexosamine levels in urine and serum, but had no effect when the urine and serum levels were normal at the start of treatment, 3) plasma hexosamine levels increased following surgical stress in adrenalectomized rats (20). The neural or humoral factors responsible for the observed changes in serum and urinary hexosamine following stress are as yet unidentified.

SUMMARY

Serum hexosamine levels increase in response to a variety of acute clinical conditions. This increase was demonstrated following myocardial infarctions, surgical operations, and an acute attack of gout. In the case of gout an increase in the alpha-globulin hexosamines was shown to account for the observed increase in total serum hexosamine.

Excretion of urine hexosamine increased following surgical operations (11) and following an attack of gout.

Corticotropin or cortisone administration was followed by a reduction in the abnormally elevated serum hexosamine levels in rheumatic fever, rheumatoid arthritis, and lupus erythematosus. Corticotropin had no significant effect in five subjects in whom the serum hexosamine levels were normal before treatment. A patient with Addison's disease was able to increase the serum hexosamine level in response to stress.

Corticotropin had no significant effect where the urinary hexosamine excretion was normal before treatment but appeared to diminish the excretion in a case in which the hexosamine had been increased.

The stress-hexosamine response in the serum and urine does not appear to be mediated by the adrenal cortex.

ACKNOWLEDGMENTS

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REFERENCES

1. West, R., and Clarke, D. H., The concentration of glucosamine in normal and pathological sera. J. Clin. Invest., 1938, **17**, 173.
2. Boas, N. F., and Soffer, L. J., The effect of adrenocorticotropic hormone and cortisone on the serum hexosamine level in acute disseminated lupus erythematosus. J. Clin. Endocrinol., 1951, **11**, 39.
3. Schlamowitz, S. T., DeGraff, A. C., and Schubert, M., Studies of tissue response to injections of enzymes. II. Changes in rabbit plasma and tissue hexosamine induced by a single subcutaneous injection of trypsin. Circulation, 1950, **1**, 822.
4. Boas, N. F., and Peterman, A. F., Effects of age, food intake, and stress on plasma hexosamine levels in the rat. Proc. Soc. Exper. Biol. & Med., 1953, **82**, 19.
5. Boas, N. F., Method for the determination of hexosamines in tissues. J. Biol. Chem., 1953, **204**, 553.
6. Videbaek, A. A., Asboe-Hansen, G., Astrup, P., Faber, V., Hamburger, CHR., Schmith, K., Sprechler, M., and Brøchner-Mortensen, K., Effect of ACTH and cortisone on rheumatic fever. Acta endocrinol., 1950, **4**, 245.
7. Astrup, P., and Grüner, A., Urinary excretion of hexosamines and glucuronic acid during treatment with ACTH. Scandinav. J. Clin. & Lab. Invest., 1951, **3**, 197.
8. Durrum, E. L., A microelectrophoretic and microionophoretic technique. J. Am. Chem. Soc., 1950, **72**, 2943.
9. Kunkel, H. G., and Tiselius, A., Electrophoresis of proteins on filter paper. J. Gen. Physiol., 1951, **35**, 89.
10. Rosenberg, C., and Schloss, B., Plasma hexosamine levels in acute rheumatic fever. Am. Heart J., 1949, **38**, 872.
11. Werner, I., On regeneration of serum polysaccharide and serum proteins in normal and intoxicated rabbits. Acta physiol. Scandinav., 1949, **19**, 27.
12. Gjessing, E. C., and Chanutin, A., An electrophoretic study of plasma and plasma fractions of normal and injured rats. J. Biol. Chem., 1947, **169**, 657.
13. Winzler, R. J., and Smyth, I. M., Studies on the mucoproteins of human plasma. II. Plasma muco-

protein levels in cancer patients. J. Clin. Invest., 1948, **27**, 617.
14. Mehl, J. W., Humphrey, J., and Winzler, R. J., Mucoproteins of human plasma. III. Electrophoretic studies of mucoproteins from perchloric acid filtrates of plasma. Proc. Soc. Exper. Biol. & Med., 1949, **72**, 106.
15. Sahlstedt, A. V., Die Schleimsubstanz des Pferdeharns. Scandinav. Arch. f. Physiol., 1916, **33**, 183.
16. Kobayasi, T., Biochemical studies on carbohydrates. LV. Urine mucoid. J. Bioch. (Japan), 1939, **30**, 451.
17. Mörner, K. A. H., Untersuchungen über die Proteinstoffe und die eiweissfällendere Substanzen des normalen Menschenharns. Skandinav. Arch. f. Physiol., 1895, **6**, 332.
18. Boas, N. F., and Foley, J. F., In preparation.
19. Tamm, I., and Horsfall, F. L., Jr., A mucoprotein derived from human urine which reacts with influenza, mumps, and Newcastle disease viruses. J. Exper. Med., 1952, **95**, 71.
20. Boas, N. F., and Foley, J. F., Effect of thyroidectomy and hypophysectomy on plasma hexosamine levels in the rat. Endocrinology, 1955, **56**, 306.

HEPATIC COMA: THE ELECTROENCEPHALOGRAPHIC PATTERN

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Recent advances in our knowledge of the neurophysiologic mechanisms underlying sleep and experimental states of coma (1) suggest the need for a neurophysiologic approach to the many complex clinical syndromes associated with disturbance of consciousness. The coma syndrome associated with hepatic disease and usually known as hepatic coma was chosen for investigation since it presents a good example of toxic coma without gross structural change in the brain. The rapid changes in level of consciousness which frequently occur in this syndrome make it particularly susceptible to experimental study.

The observations described below represent an effort to analyze such electroencephalographic (EEG) patterns as may be observed in patients with various forms of hepatic disease before, during, and after the clinical state known as hepatic coma.

MATERIAL AND METHODS

The patients to be described fall into two categories. The first is a series of 8 patients in whom there was clear evidence of hepatic disease (cirrhosis) which, on a clinical estimate, might be expected to culminate in hepatic coma. At the time of recording, these patients were in a normal mental state. This series of patients formed a control group to evaluate the EEG effect of hepatic disease without coma. The second category consists of a group of 13 patients in whom a state of hepatic coma developed.

Owing to the grave clinical condition of the patients in coma it was felt advisable to make recordings in the patient's room. Conventional solder disc electrodes were used, the placement being that shown in Figure 1, and in the early cases the recordings were made on a specially constructed 4-channel, portable inkwriting electroencephalograph. More recently, recordings have been made on a standard 8-channel, Grass, inkwriting oscillograph. The state of consciousness of the patient was evaluated throughout the recording. The effect of arousal stimuli, such as a loud clap, squeezing of the toes and pinprick, were investigated. In most instances, photic stimulation by means of strobatron light (250,000 foot candles) was carried out, the light being held 1 foot from the patient's eyes. The patients in the control group underwent a standard EEG recording using a Grass 8-channel instrument. In all instances the mental state of the patient was classified on the basis of the following terms: 1) alert, 2) confused, 3) semicoma, and 4) coma. The semicomatose patients were out of contact with their environment and spoke only a few, usually inappropriate, monosyllables. Comatose patients were those who showed little spontaneous movement and no response to noxious stimuli.

[1] The Mayo Foundation, Rochester, Minnesota, is a part of the Graduate School of the University of Minnesota.

RESULTS

Control group

The EEG findings in the group of patients with hepatic disease in the absence of mental changes are shown in Table I. An alpha rhythm was recognizable in all cases and in no instance were the EEG changes considered to be beyond normal limits. A comparison of Tables I and II indicates that the kind of hepatic involvement in the cirrhotic group without mental changes was essentially similar to that found in the group exhibiting coma (Table III). It can be concluded that the existence of hepatic disease *per se* in the absence of mental change is not associated with any detectable EEG abnormality.

Hepatic coma

The EEG changes seen in patients showing various grades of hepatic coma are summarized in Table II. In the early phases of this investigation it became apparent that at certain stages of hepatic coma, the occurrence of an unusual kind of discharge became evident in the EEG. This discharge, which we have called the "triphasic wave," was at first mistaken for an eyeblink artifact, which it resembles very closely. However, it is not difficult to eliminate eyeblink artifact as a cause of these waves since blinking is relatively

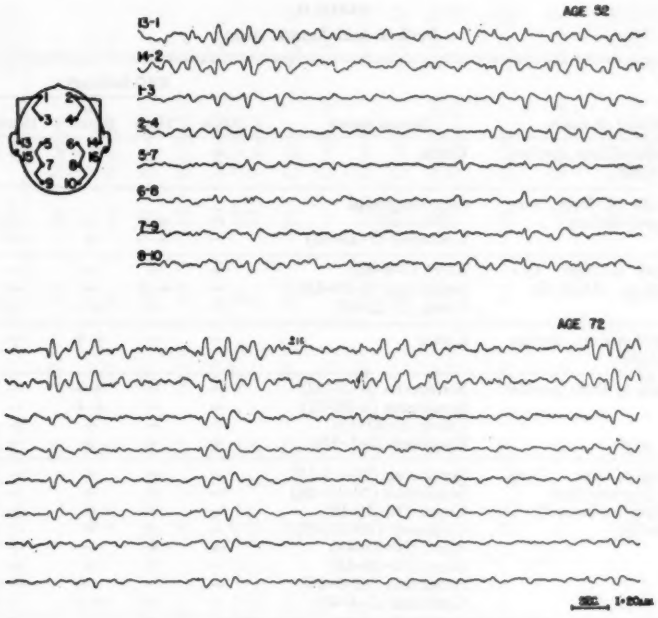

FIG. 1. EEG CHANGES IN HEPATIC COMA

Case 12, above, and case 13, below, illustrate typical groups of triphasic waves with periods of relative quiescence.

TABLE I

Patients who had cirrhosis without mental change

Case	Age *years*	Clinical diagnosis	EEG findings*			
			Alpha	Theta	Triphasic	Delta
1	41	Cirrhosis of liver, hemorrhage from esophageal varices. Alcoholic	+	–	–	–
2	47	Portal cirrhosis of liver, ascites. Alcoholic	++	–	–	–
3	41	Portal cirrhosis of liver. Alcoholic	+	–	–	–
4	57	Portal cirrhosis of liver, ascites. Alcoholic	+	–	–	–
5	49	Cirrhosis of liver, ascites. Alcoholic	+++	–	–	–
6	55	Cirrhosis, ascites. Left hydrothorax	++	–	–	–
7	35	Obstructive jaundice, biliary cirrhosis	+++	–	–	–
8	48	Postnecrotic cirrhosis	+	–	–	–

* – indicates absence.
+, ++, +++ indicate presence on a scale of 1 to 3, depending on persistence of discharges.

TABLE II

Patients with hepatic coma

Case	Age years	Clinical diagnosis	Mental status	EEG findings*				Survival after test
				Alpha	Theta	Triphasic	Delta	
9	66	Cirrhosis of liver, ascites. Alcoholic	Coma	−	−	++	−	2 days
10	51	Cirrhosis of liver, esophageal varices	Alert-forgetful (7-14-52)	−	++	−	−	6 days
			Confused (7-25-52)	−	++	+	−	
11	43	Cirrhosis of liver. G.I. bleeding. Alcoholic	Alert (7-6-48)	+	+	−	−	Few hours
			Semicoma (7-19-48)	−	++	+	−	
			Coma (7-22-48)	−	+	++	−	
12	52	Cirrhosis of liver, ascites. Alcoholic	Coma	−	−	++	+	2 days
13	72	Cirrhosis of liver, ascites	Semicoma (6-27-52)	−	−	++	−	2½ months
			Semicoma (6-28-52)	−	−	++	−	
			Coma (6-30-52)	−	−	+	+	
			Confused (7-2-52)	+	+	+	−	
14	64	Cirrhosis of liver. Cerebral degeneration (Wilson's disease?). Alcoholic	Semicoma (10-13-48)	−	+	+	−	6 weeks
			Semicoma (10-19-48)	−	+	+	+	
			Coma (10-21-48)	−	+	+	+	
			Confused (10-22-48)	−	+	+	−	
			Alert (10-25-48)	+	+	−	+	
			Alert (10-26-48)	−	+	+	+	
			Semicoma (11-9-48)	−	−	−	+	
			Confused (3-4-49)	−	+	++	−	
15	76	Biliary cirrhosis. Carcinoma of pancreas causing obstruction of bile duct	Coma	−	−	+	++	1½ hours
16	33	Biliary cirrhosis secondary to common-duct stricture	Semicoma (11-29-52)	−	+	+++	−	1 day
			Alert (12-2-52)	+++	−	−	−	
			Coma (12-9-52)	−	−	−	+	
17	46	Biliary cirrhosis secondary to common-duct stricture. Ascites	Coma	−	−	+++	−	5 days
18	42	Homologous serum hepatitis	Coma (4-20-49)	−	+	+	+	2 days. No EEG change with 2 cc. campolon I.V.
			Coma (4-25-49)	−	+	+++	−	
			Semicoma (4-26-49)	−	++	++	+	
			Confused (4-29-49)	+	+	−	−	
19	63	Infectious hepatitis	Alert (2-27-48)	+	−	−	+	2 days
			Alert (3-1-48)	+	−	−	+	
			Alert (7-12-48)	+	−	−	+	
			Coma (8-17-48)	−	−	++	−	
20	17	Postnecrotic cirrhosis. Chronic ulcerative colitis	Coma	−	−	−	+++	12 hours
21	55	Metastatic carcinoma of liver from breast	Coma	−	−	++	+	5 days

* − indicates absence.
+, ++, +++ indicate presence on a scale of 1 to 3, depending on persistence of discharges.

rare in comatose patients and the detailed properties of the waves, such as the fronto-occipital delay, could not be explained on the basis of eye movement. The properties of these waves will now be considered.

Triphasic waves

Two recordings illustrating the form and distribution of the triphasic waves are shown in Figure 1. In both instances triphasic waves are most clearly defined in the frontal and sylvian

TABLE III

Pertinent laboratory data in patients with hepatic disease

Case	Diagnosis	Alcoholic	Total serum bilirubin *mg./100 cc.*	Serum albumin *gm. %*	Serum globulin *gm. %*	Sulfobromophthalein retention %	Thymol turbidity *units*	Cephalin floc.	$ZnSO_4$ turbidity *units*	Alkaline phosphatase *units*	Prothrombin time (Quick) *sec.*	Cholesterol *mg./100 cc.*	Cholesterol esters *mg./100 cc.*	Urea *mg./100 cc.*	Mental status	Date of laboratory work done
1	Portal cirrhosis, esophageal varices	Yes	2.4	3.0	3.1	30	4	4+	11	—	24	160	56	20	Normal	—
2	Portal cirrhosis, ascites	Yes	1.0	2.7	2.2	15	1.8	2+	17	4.4	24	160	75	—	Normal	—
3	Portal cirrhosis	Yes	0.7	4.5	3.1	8	2	2+	—	4.3	26	127	64	48	Normal	—
4	Portal cirrhosis, ascites	Yes	8.9	3.32	3.23	—	3	3+	—	12	29	210	90	32	Normal	—
5	Portal cirrhosis, ascites	Yes	4.6	2.1	5	—	11.5	—	67	6	27	117	40	15	Normal	—
6	Portal cirrhosis, ascites	No	.7	3.9	2.4	8	—	—	—	—	20	190	102	20	Normal	—
7	Biliary cirrhosis	No	.9	3.3	1.7	6	2	2+	—	8	21	117	64	22	Normal	—
8	Postnecrotic cirrhosis	No	8.5	2.4	5	—	23	4+	—	12.9	23	—	—	22	Normal	—
9	Portal cirrhosis, ascites	Yes	24.4	2.1	3.4	—	2	2+	11	18	32	87	24	146	Coma	6–20–48
10	Portal cirrhosis, esophageal varices	No	.9	2.3	3.5	26	9	3+	—	—	23	—	—	—	Confused	7–14–52
11	Portal cirrhosis, esophageal varices	Yes	11.4	2.5	3.6	—	4.5	—	28	9.7	23	230	64	12	Coma	7–19–48
12	Portal cirrhosis, ascites	Yes	21.2	2.1	2.3	—	1.5	—	—	11.6	39	187	32	93	Coma	8–11–51
13	Portal cirrhosis, ascites	No	7.2	2.5	3.9	38	21	—	—	10.5	19	267	139	58	Semicoma	6–27–52
14	Cirrhosis, liver	Yes	20	3.2	4	—	4	—	18	16	30	83	20	26	Semicoma	11–9–48
15	Cancer, head of pancreas, biliary cirrhosis	No	24.9	—	—	—	5	2+	—	9.7	19	134	24	144	Coma	6–27–52
16	Common duct stricture, biliary cirrhosis	No	8	2.3	4	—	5	3+	—	—	26	112	—	10	Semicoma	12–2–52
17	Intrahepatic duct stone, biliary cirrhosis	No	19	2.1	3.5	—	4	4+	28	—	26	—	—	18	Coma	7–24–52
18	Serum hepatitis	No	20.7	3.5	1.3	—	1	4+	4	—	47	75	20	106	Semicoma	4–20–49
19	Infectious hepatitis	No	38.6	2.5	2.1	—	7	4+	9.6	7.1	36	190	44	36	Coma	8–17–48
20	Postnecrotic cirrhosis, chronic ulcerative colitis	No	2.1	1.8	3.3	22	12	3+	—	5.9	24	134	67	—	Coma	5–2–52
21	Metastatic carcinoma of liver from breast	No	1.8	—	—	40	—	—	—	—	22	—	—	46	Coma	12–20–54

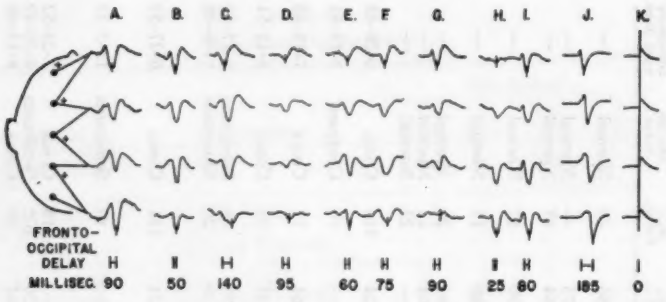

FIG. 2. VARIABILITY IN DELAY AND DISTRIBUTION OF THE TRIPHASIC WAVES

A, patient, 12; patient, case 13; J, synthetic waves from a model. In all cases the delays have been corrected for pen alignment and arc distortion of the trace.

areas, the amplitude falling to a relatively low value in the occipital region. The main deflection is downward, indicating a surface positive change. The main deflection is usually preceded and followed by low-amplitude negative deflections giving the whole complex a triphasic contour. The shape of the complex varies owing to changes in the relative prominence of the three waves.

There is a tendency for the occipital discharges to lag slightly in time in relation to the frontal discharges. This is well shown in Figure 2, in which the fronto-occipital delay has been measured for a series of waves belonging to various patients (A to I). The occipital regions show a delay varying from 25 to 140 milliseconds, following the peak of the main positive frontal discharge. These delays were not so clearly distinguishable in monopolar recordings. There is some variation in the contour of the discharge in different areas of the cortex and in a few instances (I) the amplitude is maximal in the occipital area. At other times the wave almost disappears before reaching the occipital area (G). Another variant is the wave which appears to arise in the sylvian regions and sweep posteriorly without involving the frontal regions appreciably. A tendency for the discharge to broaden as it migrates toward the occipital area is also frequently observed (F). The variability described above is usually bilateral so that a high degree of symmetry between recordings from the two hemispheres is maintained. In addition to this there is usually bilateral synchrony of the triphasic waves within a few milliseconds.

The mechanism by which the triphasic waves are generated by the cortex is obscure, but evidence of delay in the peak of the discharge in the occipital areas, as compared with the frontal, naturally raises the question of a traveling wave of positivity sweeping the cortex from the frontal to the occipital regions. That such a moving electrical field will produce a deflection roughly comparable to the triphasic waves of the human being is shown in J (Figure 2), which is a recording from a model consisting of a saline bath in which

TABLE IV

EEG stages in hepatic coma

	EEG pattern		
	Theta (4–7 c/s)	Triphasic	Delta (0.5–3 c/s)
Alpha	Present but may be partly disorganized	Usually absent	Absent
Mental status	Range from normal to mild confusion with forgetfulness and irrational behavior	Range from disorientation and confusion to semicoma	Semicoma to deep coma
Prognosis	May recover	May recover	Usually terminal

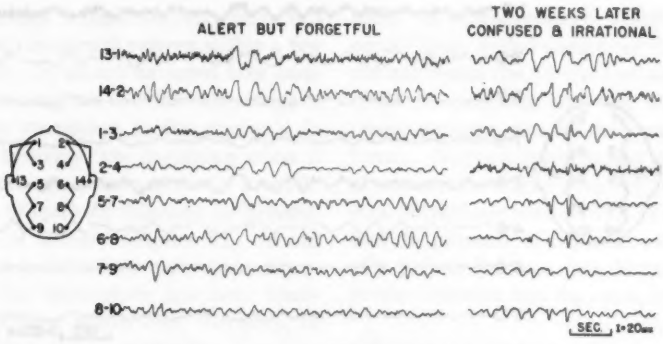

FIG. 3. CASE 10: THETA PHASE OF HEPATIC COMA, WITH PROGRESSION TO TRIPHASIC PHASE SHOWN ON THE RIGHT

a positive pole is swept past a series of electrodes representative of their position on the scalp. A conduction rate for this wave of about 1.5 meters per second would be required to give the observed delays, a value which is well within the observed conduction rates for the central tracts in animals.

A further possibility is that the primary disturbance is traveling at a subcortical (thalamic ?) level and that the changes in potential field observed on the cortical surface are merely the reflection of the primary disturbance (*via* thalamocortical relays) on to the cortical surface. The evidence at hand does not allow us to distinguish between these two hypotheses.

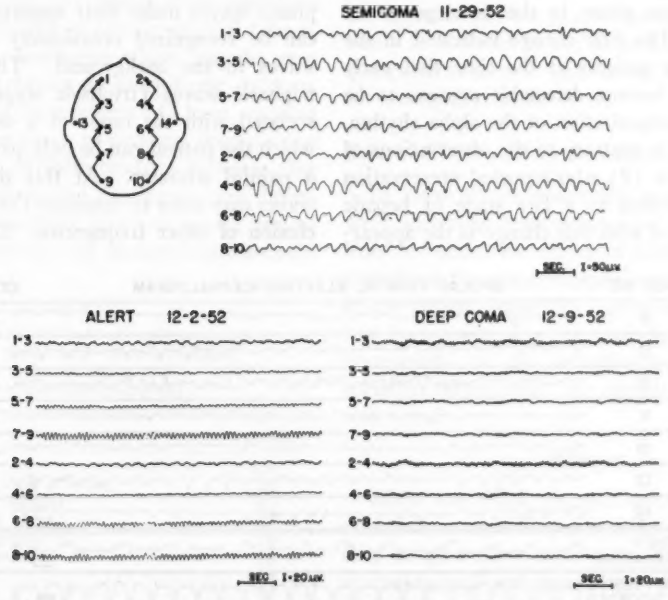

FIG. 4. CASE 16: CHANGES IN EEG RECORDINGS FROM A CASE OF HEPATIC COMA

Triphasic waves are closely clustered in the recording on November 29, 1952, though their presence is clearly evident in tracings from electrodes 1–3 and 2–4.

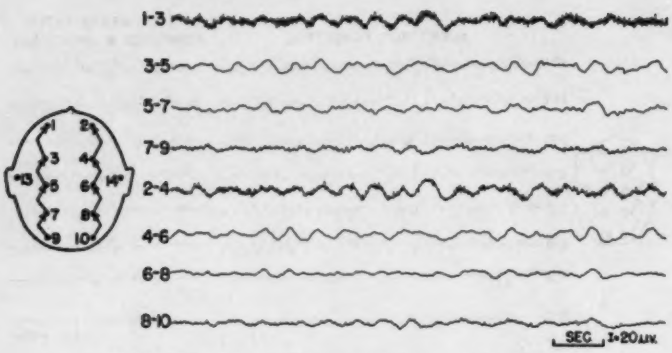

FIG. 5. CASE 20: SLOW IRREGULAR WAVES OF THE DELTA STAGE IN A CASE OF POSTNECROTIC CIRRHOSIS

The patient died 12 hours after the recording was made.

1. Occurrence of triphasic waves. While reference to Table II shows that triphasic waves were observed in all but one of the patients with disturbed consciousness, multiple recordings were frequently necessary to demonstrate their presence. A review of the findings indicates that they occur at a certain phase in the development of hepatic coma. The first change indicated in the records of these patients at the time that early mental changes become detectable appears to be slowing and disorganization of the alpha rhythm, a finding which is contrary to the observations of Adams and Foley (2) who reported preservation of the alpha rhythm to a late stage of hepatic coma. Associated with this change is the appearance of waves of a frequency of 4 to 7 per second from all areas of the cortex. This may be called the "theta phase" (Table IV and Figure 3), and at this stage the patient is usually slightly disoriented with respect to time and place. With further deterioration in the clinical condition, triphasic waves make their appearance so that they can be recognized occasionally among the slow waves in the background. The appearance of triphasic waves (triphasic stage) is usually associated with the onset of a deeper coma from which the patient can be only partially aroused by a painful stimulus. At this time the triphasic waves may come to dominate the record to the exclusion of other frequencies. In some instances

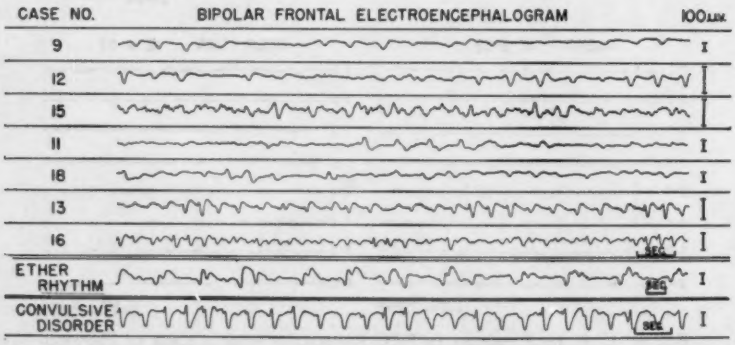

FIG. 6. SOME TYPICAL TRACINGS SHOWING TRIPHASIC WAVES IN HEPATIC COMA, WITH RECORDINGS FROM A PATIENT UNDERGOING ETHER ANESTHESIA AND FROM A PATIENT WITH A CONVULSIVE DISORDER FOR COMPARISON

These are bipolar recordings from frontomotor regions.

the patient may make a rapid recovery from this stage with a return to normal pattern within a few hours (Figure 4). It should be noted how complete the EEG recovery can be without a vestige of abnormal activity remaining in the tracing. In the event of no recovery, the deepening coma is accompanied by the appearance of slower arrhythmic delta waves (Figure 5) followed by generalized suppression of activity (Figure 4). In our limited experience patients have not regained consciousness once the delta phase has been established for several days, which may, therefore, be regarded as having a grave prognosis.

2. Triphasic waves in other clinical conditions. The triphasic wave cannot be regarded as being entirely characteristic of the EEG in hepatic coma since its presence is confined to certain phases of the illness. In addition, somewhat similar discharges may be seen in such diverse conditions as ether anesthesia (Figure 6) and in rare instances in convulsive disorders. It has not been our experience that the discharge resembles the spike and wave of petit mal as suggested by Foley, Watson, and Adams (3) though there may be a fairly close resemblance to certain atypical bilaterally synchronous discharges which may be seen in the electroencephalogram of epileptic children. Cobb, Martin, and Pampiglione (4) have reported triphasic waves in cases of cerebral lipidosis but careful inspection of their tracings indicates that the polarity, duration, and distribution are different from those described here. A recent review of the EEG records of 345 patients with disturbances of consciousness (confusion, semicoma, coma) from varied causes, as shown in Table V, did not reveal the presence of any discharges that could be confused with the well-developed triphasic patterns seen in the patients with hepatic coma. Workers investigating the EEG in coma of nonhepatic origin have not reported the presence of triphasic waves (5). It may be concluded that the presence of triphasic waves in the record of a patient in coma may be taken as very suggestive evidence that the coma is hepatic in origin.

TABLE V

Diagnostic categories of 345 patients with a mental state of confusion, semicoma or coma but without triphasic waves (chronologic unselected group)

Diagnosis	Patients
Cerebral tumor	129
Degenerative disease of brain	50
Cerebrovascular disease	43
Cerebral trauma	27
Encephalitis, meningitis	20
Subdural hematoma	18
Cerebral infarct	18
Psychosis	12
Organic psychosis	9
Convulsive disorder, postictal	8
Drug intoxication	5
Diabetes	4
Anoxic encephalopathy	1
Uremia	1

Effect of sensory stimulation

The effect of a painful arousal stimulus was determined in many patients in the various stages of hepatic coma. In the triphasic stage, the patient usually showed some response such as the withdrawal of a limb, but no consistent change in the EEG pattern during stimulation was observed. Likewise, photic stimulation was usually ineffective in producing a change in the triphasic pattern, and no response at the flash frequency used was seen in any case. The lack of response of the triphasic pattern to afferent stimulation is certainly in contrast to the responsiveness of abnormal patterns seen in some other varieties of coma, for example, postictal coma.

Effect of liver extract on electroencephalogram

In view of the possibility that these patients were suffering from some kind of deficiency of liver secretion, the effect of liver extract (campolon) on the electroencephalogram was observed in one patient (case 18). After a control tracing had been made, 2 cc. of campolon was given intravenously and the recording was continued for a further 90 minutes. No clinical or EEG change was noted.

COMMENT

While the EEG changes described cannot be regarded as indicating a specific pathologic state, they did give some clues to the likely mechanisms of the mental and neurologic symptoms of hepatic coma. The strict bilateral synchrony of the electrical discharges in the triphasic stage would suggest the participation of a diffuse synchronizing network, such as the thalamocortical reticular system. The rapid and complete reversibility

of the EEG changes, at least in the triphasic stage, argues in favor of a chemical rather than a structural change in the region of the brain primarily involved. Furthermore, some chemical substances, such as ether, can produce electrical changes resembling those of hepatic coma.

The existence of some metabolic relationship between the liver and the brain has been suspected for more than a century. The demonstration by Geiger and Magnes (6) that a substance contained in the liver (or liver extract) is necessary for the persistence of electrical activity in the artificially perfused brain provided a physiologic basis for this relationship. More recently, Taylor (7) has attributed this survival-prolonging effect of liver extract upon EEG activity in the isolated brain preparation to its property of reducing permeability of the brain cell to glucose.

The data reported here seem to indicate that the particular kind of hepatic involvement (portal or biliary cirrhosis, or metastasis to the liver as in case 21) is unimportant so far as the tendency to produce characteristic EEG changes is concerned. This would suggest that the EEG changes result from some factor common to various types of hepatic pathology.

Independent evidence has recently been presented which suggests that various chemical substances are the responsible agents in the production of hepatic coma. Walshe (8) has reported an increased glutamine concentration in the cerebrospinal fluid of three patients who were in hepatic coma. More recently a number of workers have suggested that hepatic coma is related to metabolism of ammonia. Gabuzda, Phillips, and Davidson (9) described a syndrome indistinguishable from hepatic coma occurring in patients with cirrhosis given certain ammonia-containing cation exchange resins. The cause of the reaction was not apparent, but it was suggested that the effects were due to ammonia intoxication. The EEG alteration seen in one of their patients was said to simulate those described in hepatic coma. Later, Phillips, Schwartz, Gabuzda, and Davidson (10) demonstrated that apparently identical untoward effects can be provoked in patients with cirrhosis of the liver by administration of ammonium chloride, diammonium citrate, urea or dietary protein. The published illustration of the EEG tracings taken at the peak of the clinical reaction to these substances showed the nonspecific slow wave which may be seen in coma due to a number of different causes. No triphasic waves were recognizable in these tracings. Although increase in blood ammonia was suspected as being the cause of the reaction, the correlation between the concentration of blood ammonia and the abnormal signs observed was not consistent enough to establish a relation.

More recently, McDermott and Adams (11) have described a patient with an Eck fistula whose mental condition seemed to be related to the level of blood ammonia, and who presented episodes of stupor when the intake of substances related to ammonia metabolism was changed artificially. The described changes in the EEG again appear to be nonspecific, and in the absence of published tracings it is impossible to be certain whether or not triphasic waves were present at any stage.

It is evident that an increasing difficulty in these cases is that of relating induced "hepatic-comalike" syndromes now encountered in both clinical and animal work with the genuine clinical hepatic-coma syndrome. Electroencephalographic data should contribute useful evidence in establishing these relationships in addition to their diagnostic and perhaps prognostic value in individual patients.

SUMMARY

In the electroencephalograms of 12 patients in various stages of hepatic coma the following stages have been recognized as the depth of coma increased: 1) theta stage, with diffuse waves of a frequency of 4 to 7 per second, 2) triphasic stage characterized by diffuse bilaterally synchronous triphasic waves whose maximal deflection is surface positive, and 3) delta stage in which random arrhythmic waves with little bilateral synchrony dominate the record. A control group of 8 patients who had hepatic cirrhosis and impairment of hepatic function, as measured by laboratory means, that were essentially as great as that of the 13 patients, but who had no mental change, showed EEG recordings that were within normal limits.

Patients in the theta stage are usually moderately confused, while those in the triphasic and delta stages are usually semicomatose or completely unresponsive.

Some evidence from latency measurements suggests that the triphasic wave is a conducted disturbance. Waves somewhat similar to the triphasic discharges have been seen in other conditions such as ether anesthesia and convulsive disorders but not in patients in coma from other causes.

It is suggested that in addition to being clinically useful from the diagnostic and perhaps prognostic points of view, the EEG may assist in relating experimental coma syndromes to genuine hepatic coma.

REFERENCES

1. Magoun, H. W., The ascending reticular activating system. A. Research Nerv. & Ment. Dis., Proc., 1952, **30**, 480.
2. Adams, R. D., and Foley, J. M., The neurological disorder associated with liver disease. A. Research Nerv. & Ment. Dis., Proc., 1953, **32**, 198.
3. Foley, J. M., Watson, C. W., and Adams, R. D., Significance of the electroencephalographic changes in hepatic coma. Tr. Am. Neurol. A., 1950, **75**, 161.
4. Cobb, W., Martin, F., and Pampiglione, G., Cerebral lipidosis: An electroencephalographic study. Brain, 1952, **75**, 343.
5. Li, C.-L., Jasper, H., and Henderson, L., Jr., The effect of arousal mechanisms on various forms of abnormality in the electroencephalogram. Electroencephalog. & Clin. Neurophysiol., 1952, **4**, 513.
6. Geiger, A., and Magnes, J., The isolation of the cerebral circulation and the perfusion of the brain in the living cat. Am. J. Physiol., 1947, **149**, 517.
7. Taylor, R. M., Technic for perfusion of the completely isolated brain in the living cat *in* Milbank Memorial Fund, 27th Annual Conference: The Biology of Mental Health and Disease, New York, Paul B. Hoeber, Inc., 1952, pp. 48–53.
8. Walshe, J. M., Observations on the symptomatology and pathogenesis of hepatic coma. Quart. J. Med., 1951, n.s. **20**, 421.
9. Gabuzda, G. J., Jr., Phillips, G. B., and Davidson, C. S., Reversible toxic manifestations in patients with cirrhosis of the liver given cation-exchange resins. New England J. Med., 1952, **246**, 124.
10. Phillips, G. B., Schwartz, R., Gabuzda, G. J., Jr., and Davidson, C. S., The syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances. New England J. Med., 1952, **247**, 239.
11. McDermott, W. V., Jr., and Adams, R. D., Episodic stupor associated with an Eck fistula in the human with particular reference to the metabolism of ammonia. J. Clin. Invest., 1954, **33**, 1.

THE ELABORATION OF OSMOTICALLY CONCENTRATED URINE IN RENAL DISEASE

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During the hydropenic state, the kidney, in the interest of water conservation, excretes a urine which is osmotically more concentrated than the blood. Since the glomerular filtrate has the same osmotic pressure as the plasma, in the elaboration of an osmotically concentrated urine the renal tubules must remove from this isosmotic filtrate a certain quantity of solute free water. The quantity of water so removed from any sample of urine is given by the difference between the volume of urine required to contain the urinary solutes in an isosmotic state, or the osmolar clearance, C_{osm}, and the urine-flow, V (1). The osmolar clearance is given by the calculation $U_{osm}V/P_{osm}$, where U_{osm} and P_{osm} are the osmotic concentrations of the urine and plasma, respectively, and V is the urine flow in cc. per min. Hence the solute-free water, $T^c_{H_2O}$, removed by the tubules in concentrating the urine is

$$(1) \qquad T^c_{H_2O} = \frac{U_{osm}V}{P_{osm}} - V.$$

This concentrating operation is subject to two limitations. Zak, Brun, and Smith (2) have shown that at large urine flows induced by osmotic diuresis in the antidiuretic state, $T^c_{H_2O}$ attains an approximately constant value, $Tm^c_{H_2O}$. At lower urine flows, however, when C_{osm} is less than $Tm^c_{H_2O}$, the reabsorption of solute-free water proceeds until the urine approaches a maximal osmotic concentration, or (what is equally probable) until the osmotic U/P ratio approaches a maximal value, which in normal subjects is about 4.0 (3).

It has long been conventional to judge the concentrating operation of the kidney by the specific gravity of the urine, but specific gravity is not an appropriate index for this purpose because it does not accurately reflect the basic physiological operation of the kidney, which can only be judged in terms of the quantity of water abstracted.

In the present study, the concentrating operation, as evaluated by $T^c_{H_2O}$, has been examined in patients with acute and chronic renal disease.

METHODS

Our subjects comprise 25 patients, drawn from various services of Bellevue Hospital, with the following diagnoses: acute glomerulonephritis, 5; chronic glomerulonephritis, 14; chronic pyelonephritis, 1; Kimmelstiel-Wilson syndrome, 2; and acute renal failure, 3. All subjects were maintained on the regular hospital diet except for variations in salt intake. Fluids were withheld during 15 to 18 hours preceding the test, which was performed in the morning with the patient in a fasting state. Urine was collected by means of an indwelling catheter, and residual urine was expelled from the bladder by means of air. No washout fluid was used. Surgical sterility was maintained throughout the test.

Control urine and plasma samples were collected for the determination of inulinoid blank and oliguric osmotic U/P ratio. After priming doses of inulin (±3.0 grams) and of Pitressin® (Parke, Davis and Co.) (50 to 100 milliunits) had been administered intravenously, an infusion containing inulin and Pitressin® was established, the latter being administered at a rate of 1 milliunit per kg. of body weight per hour. The rate of infusion of inulin was adjusted to maintain a plasma concentration of ca. 25 mg. per 100 cc.

Urine was collected for three periods of 15 to 20 minutes each for the determination of the basal filtration rate and osmotic U/P ratio. Thereafter, progressively increasing osmotic diuresis was induced by administration of 10 per cent mannitol solution at a rate of 10 to 20 cc. per min. into the infusion tubing, the infusion of inulin and Pitressin® being continued throughout. Urine collection was continued for 6 to 10 periods of 10 minutes each, and blood was drawn at appropriate intervals from an antecubital vein into heparinized syringes, centrifuged immediately and the plasma stored in stoppered tubes.

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Inulin was determined by Schreiner's modification of the resorcinol method of Roe (4), and osmotic pressure of plasma and urine was determined by means of the thermistor bridge-null-point-detector unit of Bowman, Trantham, and Caulfield (5), using a Johlin freezing point apparatus (6). Urine specific gravity was determined with a clinical urinometer, if the urine volume was adequate; otherwise the specific gravity was determined by weighing the sample.

RESULTS

When the osmolar clearance, C_{osm}, approaches or is less than the maximal value of $T^c_{H_2O}$, the concentrating mechanism is not "saturated" and solute-free water reabsorption becomes limited by an approach to the limiting osmotic U/P ratio. It is therefore necessary to consider in each instance whether C_{osm} is large enough to fulfill the condition that $T^c_{H_2O}$ is maximal, or approximately so. To this end, in considering the data for each subject, C_{osm} was plotted against V, as in Figure 1, which shows a series of observations in subject No. 10. The nature of this graph is such that if the urine were consistently isosmotic with the plasma, C_{osm} would always be equal to V and all points would fall on the bisector, or isosmotic parameter (7). In so far as the data are displaced to the left of the isosmotic parameter, it is inferred (2) that solute-free water has been abstracted from isosmotic glomerular filtrate to an extent equal to the leftward, horizontal displacement (line **b** in Figure 1).

The first three points at the left of Figure 1 generate a straight line **a** which intersects the zero coordinates, and represents a constant osmotic U/P ratio—in this subject having a value of 1.90. At higher values of V, $T^c_{H_2O}$ reaches an approximately constant value, $Tm^c_{H_2O}$. In this

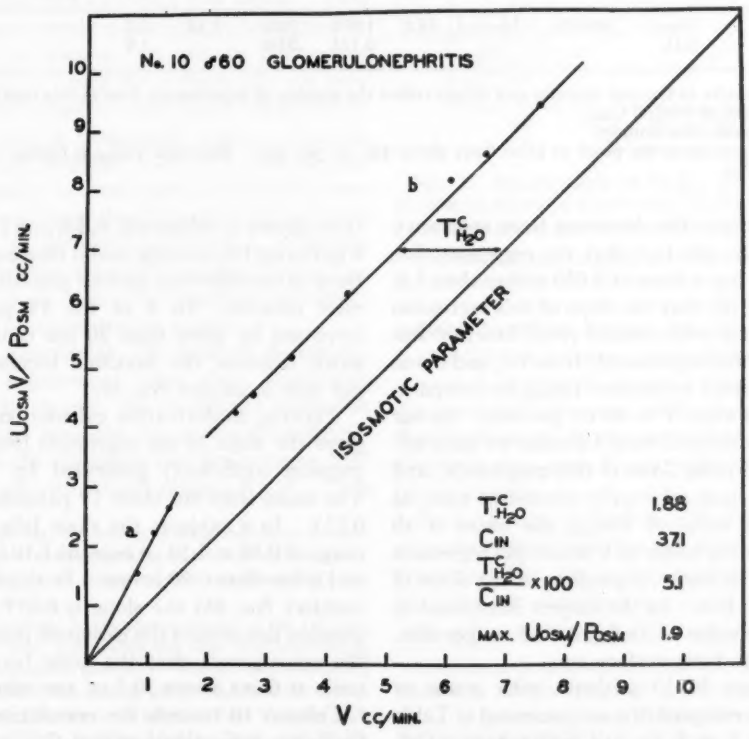

FIG. 1. URINARY CONCENTRATING PROCESS IN A 60-YEAR OLD MALE WITH CHRONIC GLOMERULONEPHRITIS

Patient studied during hydropenic state with mannitol osmotic diuresis and Pitressin® administration.

TABLE I

Osmotic diuresis during hydropenia and Pitressin® administration in 19 patients with acute or chronic glomerulonephritis

1 No.*	2 Age yrs.	3 Sex	4 Surface area sq. m.	5 Diagnosis	6 P_{osm} range mOsm./L.	7 Urine flow range cc./min.	8 C_{IN} per 1.73 sq. m. cc./min.	9 Regres. coef. (slope)	10 Correl. coef. r	11 $Tm^{c}_{H_2O}$ per 1.73 sq. m. cc./min.	12 $Tm^{c}_{H_2O}/C_{IN}$† X100	13 Maximal‡ specific gravity	14 Maximal‡ U_{osm}/P_{osm}	15 $V/C_{IN} \times 100$ (at maximal diuresis) per cent
15	8	F	1.01	Acute	305–322	2.4–8.9	88.9	1.197	.9915	6.03	6.8	—	3.06	16.8
19	7	M	0.89	Acute	267–288	3.1–8.6	78.6	1.286	.9999	6.96	8.8	1.015	3.28	15.6
30	38	F	1.82	Acute	280–288	5.4–7.9	78.6	1.159	.9099	4.90	6.2	1.028	3.10	8.9
26	37	F	1.62	Chronic	265–287	3.8–14.6	73.2	1.183	.9908	5.09	6.9	—	3.24	20.8
37	39	M	2.00	Acute	287–299	9.1–30.9	72.4	0.958	.9841	4.79	6.6	1.014	2.30	31.6
21	12	F	1.33	Chronic	304–308	4.9–12.5	71.3	1.258	.9975	5.52	7.8	—	1.87	22.1
34	35	F	1.32	Chronic	277–301	3.7–9.7	70.8	1.002	.9673	3.76	5.3	1.013	2.50	30.7
25	42	M	1.69	Acute	294–297	4.1–7.6	68.4	1.166	.9431	4.19	6.1	—	2.30	10.2
8	12	M	1.05	Chronic	299–321	2.2–15.8	62.3	1.019	.9967	3.43	5.5	1.026	2.65	15.6
5	54	M	1.95	Chronic	284–292	2.9–12.7	57.5	1.128	.9913	1.63	2.8	1.011	1.72	19.2
29	57	F	1.75	Chronic	290–305	7.7–9.6	53.6	0.905	.9999	3.18	5.9	1.018	2.01	15.8
6	29	F	1.56	Chronic	272–286	2.0–6.5	46.7	1.056	.9996	1.40	3.0	—	1.85	12.6
7	65	M	1.73	Chronic	308–317	1.9–7.0	41.0	1.003	.9988	2.15	5.2	—	2.70	15.2
38	49	F	1.56	Chronic	285–289	4.9–23.2	39.8	1.068	.9924	1.99	5.0	—	1.59	46.2
2	12	F	1.04	Chronic	296–304	2.3–6.1	38.3	1.011	.9927	1.18	3.1	1.013	1.46	26.5
10	60	M	1.74	Chronic	302–316	2.5–7.6	37.1	1.059	.9999	1.88	5.1	1.017	1.90	22.7
35	37	M	1.69	Chronic	283–306	2.2–15.5	23.0	0.877	.9905	0.44§	1.9	—	1.23	50.0
3	62	M	2.02	Chronic	301–307	1.9–3.7	14.1	0.935	.9996	0.21	1.5	1.010	1.15	21.6
32	27	M	1.90	Chronic	302–320	2.0–4.3	12.8	1.132	.9993	0.78	6.1	1.020	1.51	27.6
				Mean	290–302	3.6–11.2	54.2	1.074	.9866	3.13	5.2	—	—	—
				S.D.				0.113	.0226		1.9			

* Numbers refer to the case histories and do not reflect the number of experiments done in this study.

† Ratio based on control C_{IN}.

‡ Not on same urine samples.

§ Urine hypotonic to the blood at urine flows above 10.7 cc. per min. Recorded $Tm^{c}_{H_2O}$ is highest value observed at low urine flows.

particular subject the deviation from constancy is indicated by the fact that the regression line of C_{osm} on V has a slope of 1.059 rather than 1.0. It was found (2) that the slope of this regression line in subjects with normal renal function frequently deviated significantly from 1.0, and it was arbitrarily elected to measure $Tm^{c}_{H_2O}$ for comparative purposes when V = 20 cc. per min. In our subjects with reduced renal function we have seldom attained urine flows of this magnitude, and therefore we have arbitrarily elected to take, as the definitive value of $Tm^{c}_{H_2O}$, the mean of all values within the range of V where the regression of C_{osm} on V is linear, regardless of the slope of the regression line. In the subject illustrated in Figure 1, the value of $Tm^{c}_{H_2O}$ is 1.88 cc. per min. per 1.73 sq. m. body surface area.

Observations in 19 patients with acute or chronic glomerulonephritis are presented in Table I. Columns 1 to 5 are self explanatory. Column 6 shows the extremes of plasma osmolarity during the period of observation, and column 7 the range of urine flow involved in the calculations shown in columns 8, 9, 10, and 11. Column 8 indicates the average inulin clearance (C_{IN}) for three urine collection periods preceding the mannitol infusion. In 6 of the 19 patients, C_{IN} increased by more than 20 per cent during osmotic diuresis, the maximal increase being 37 per cent in subject No. 35.

Turning to derivative calculations, column 9 gives the slope of the regression line (or the regression coefficient) generated by C_{osm} on V. The mean slope for these 19 patients is 1.074 ± 0.113. In 9 subjects the slope falls outside the range of 0.90 to 1.10; it exceeds 1.10 in 8 subjects, and is less than 0.90 in one. In this last instance (subject No. 35) the slope is 0.877 and the regression line crosses the isosmotic parameter with the consequence that the urine becomes hypotonic at flows above 10.7 cc. per minute.

Column 10 records the correlation coefficient for linear regression between C_{osm} and V. The generally high value of this correlation coefficient was noted by Zak, Brun, and Smith who remarked that it implied both considerable relia-

bility in the individual observations and regularity in the underlying physiological process.[3]

Column 11 records the mean value of $Tm^{c}_{H_2O}$ calculated as stated above and column 12 gives the ratio $Tm^{c}_{H_2O}/C_{IN}$. This ratio is more illuminating than the absolute values of $Tm^{c}_{H_2O}$, since it reveals the relative impairment of the two functions, concentrating operation and filtration rate.

This ratio varies widely in our subjects (from 1.5 to 8.8), but only slightly more so than in normal subjects: our mean value for this ratio is 5.2 ± 1.9, as compared with a mean value of 5.1 ± 1.5 in normal subjects.[4] However, this ratio appears to vary with the underlying renal disease, as shown by Figure 2. In all subjects with an inulin clearance above 60 cc. per min. $Tm^{c}_{H_2O}/C_{IN}$ has a value of 5.3 or greater: whereas in 5 of 10 subjects with an inulin clearance below 60 cc. per min. this ratio is 3.1 or below. This suggests that in acute glomerulonephritis, and in some subjects diagnosed as chronic glomerulonephritis but in whom the disease has not progressed far as judged by reduction of the filtration rate, there may be a relatively greater reduction in glomerular activity than in concentrating power, probably in consequence of inflammatory changes or scarring of the glomerular tuft without obliteration of the subjoined tubule. Whereas in advanced glomerulonephritis, a low $Tm^{c}_{H_2O}/C_{IN}$ ratio (Nos. 2, 3, 5, 6, 35) suggests a relatively greater loss in concentrating power.

Table II records data obtained on 6 patients with renal impairment other than glomerulonephritis. In general, the results are much the same as those recorded for glomerulonephritis. The slope of the regression line lies between 0.90 and 1.10 in all cases except subject No. 9. This patient resembles subject No. 35, in that there is coincidence between a low slope (0.824) and a marked increase in filtration rate (46 per cent) during osmotic diuresis,[5] while the maximal urine flow represented 38 per cent of the simultaneous filtration rate and 55 per cent of the control filtration rate.

In 3 subjects studied shortly after an episode of acute renal failure (No. 22, mercury bichloride poisoning, No. 39, carbon tetrachloride poisoning, No. 9, transfusion reaction[6]) the ratio $Tm^{c}_{H_2O}/C_{IN}$ was low: 1.7, 2.8, and 0.8 cc. per 100

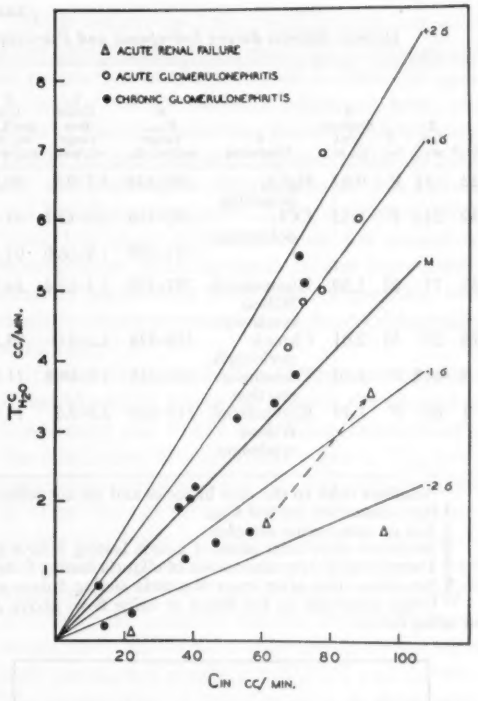

FIG. 2. RELATIONSHIP OF $Tm^{c}_{H_2O}$ TO C_{IN} IN 19 SUBJECTS WITH ACUTE OR CHRONIC GLOMERULONEPHRITIS AND 3 SUBJECTS AFTER ACUTE RENAL FAILURE

The data are superimposed on a curve representing the mean normal ratio ± 1 and 2σ.

[3] We may add that one source of error ordinarily present in other clearance determinations, namely the independent measurement of the filtration rate, is not involved in the measurement of C_{osm}.

The error involved in the measurement of the freezing point, <1 per cent, is probably less than that involved in colorimetric procedures generally. Lastly, errors involved in urine collection and timing affect C_{osm} and V almost proportionally. However, we must reaffirm that the high correlation coefficient implies, as Zak, Brun, and Smith stated, impressive regularity in the underlying physiological processes.

[4] We have calculated the mean value of this ratio from the data in Table I of Zak, Brun, and Smith.

[5] Exclusive of this subject and No. 35, the greatest increase in filtration observed in any subject was 23 per cent. In general, however, we see no correlation between the regression coefficient and the maximal change in filtration rate during osmotic diuresis.

[6] It must be noted that the slope of the regression line in this subject is below 1.0 (0.824) and the figure 0.18 represents the magnitude of $T^{c}_{H_2O}$ at the lowest urine flows.

TABLE II

Osmotic diuresis during hydropenia and Pitressin® administration in six patients with renal diseases

1 No.*	2 Age yrs.	3 Sex	4 Surface area sq. m.	5 Diagnosis	6 P_{osm} range mOsm./L.	7 Urine flow range cc./min.	8 C_{IN} per 1.73 sq. m. cc./min.	9 Regres. coef. (slope)	10 Correl. coef. r	11 $Tm^{e}_{H_2O}$ per 1.73 sq. m. cc./min.	12 $Tm^{e}_{H_2O}/C_{IN}$† ×100	13 Maximal‡ specific gravity	14 Maximal‡ U_{osm}/P_{osm}	15 $V/C_{IN}\times 100$ (at maximal diuresis) per cent
22	4§	F	0.62	$HgCl_2$ poisoning	304–334	5.7–9.5	96.0	1.101	0.9999	1.59	1.7	1.012	2.52	26.0
39	24‖	F	1.52	CCl_4 poisoning	302–326	2.8–12.4	61.1	1.061	0.9980	1.71	2.8	—	1.54	22.5
					272–299	1.9–16.0	91.3	0.999	0.9942	3.59	3.9	—	2.9	20.0
33	71	M	1.50	Kimmelstiel-Wilson syndrome	307–320	2.3–10.4	34.0	1.046	0.9713	2.06	6.0	1.018	1.87	24.7
18	29	M	2.01	Chronic pyeloneph.	319–324	4.1–9.0	25.5	0.961	0.9780	1.20	4.7	1.010	1.47	27.9
9	44¶	F	1.56	Transfusion reaction	316–335	1.0–10.8	21.6	0.824	0.9999	0.18**	0.8	1.011	1.14	38.0
4	60	F	1.49	Kimmelstiel-Wilson syndrome	310–330	2.0–3.2	11.9	1.041	0.9968	0.45	3.8	1.010	1.20	17.5

* Numbers refer to the case histories and do not reflect the number of experiments done in this study.
† Ratio based on control C_{IN}.
‡ Not on same urine samples.
§ Seventeen days after onset of anuria lasting 2 days and oliguria lasting 2 days.
‖ Twenty-eight days after onset of oliguria lasting 9 days. Second series of observations performed 5 months later.
¶ Seventeen days after onset of anuria lasting 5 days and oliguria lasting 9 days.
** Urine hypotonic to the blood at urine flows above 2 cc. per min. Recorded $Tm^{e}_{H_2O}$ is largest value observed at low urine flows.

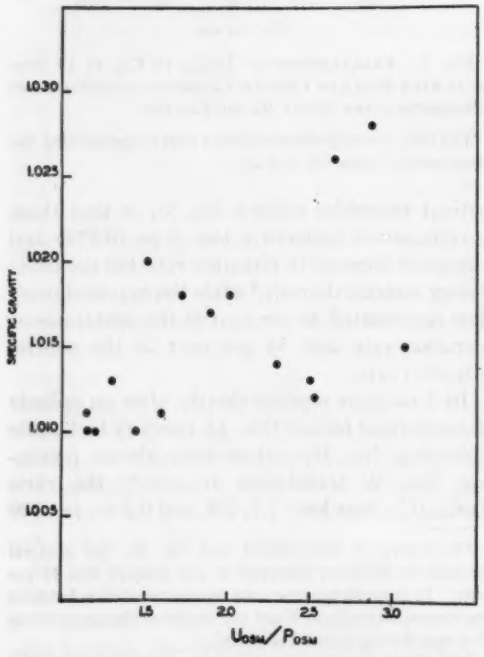

FIG. 3. COMPARISON OF URINARY SPECIFIC GRAVITY AND OSMOTIC U/P RATIO AS DETERMINED BY THE CRYOSCOPIC METHOD

cc. of filtrate (see Figure 2), as compared to 1.8, 2.6, and 2.7, the lowest ratios observed in normal subjects, and 1.5 and 1.9, the lowest ratios observed in subjects with glomerulonephritis. In subjects No. 22 and 39, however, the filtration rate was above 60 cc. per min., and these two subjects, if not subject 9, bear out the presumption that concentrating capacity may be markedly impaired after acute renal failure, even at a time when the filtration rate has substantially recovered. Observations were repeated in subject No. 39 five months after recovery from acute renal failure. At this time the filtration rate had increased from 61 to 91 cc. per min., $Tm^{e}_{H_2O}$ from 1.7 to 3.6 cc. per min., and the $Tm^{e}_{H_2O}/C_{IN}$ ratio from 2.8 to 3.9, indicating further recovery of tubular function. In fact, the latter increased relatively more than did the filtration rate.

Column 13 records the maximal urinary specific gravity as determined by the usual clinical concentration test, *i.e.*, after 15 to 18 hours' abstention from fluid. This measurement was made on the initial urine collected at the beginning of our observations. The ratio $Tm^{e}_{H_2O}/C_{IN}$ and maximal specific gravity are poorly correlated: subjects with ratios well above the mean

normal value of 5.1 may have a maximal specific gravity of only 1.011 to 1.015.

Column 14 gives the maximal osmotic U/P ratio observed in each patient. Figure 3 shows the relationship between specific gravity and osmotic U/P ratio in the same urine samples, with selection of those samples showing the maximal specific gravity. The gross lack of correlation in Figure 3 is obvious. In the specific gravity range of 1.010 to 1.012, ordinarily interpreted as indicating loss of "concentrating ability," one finds osmotic U/P ratios as high as 2.5; on the other hand, in one subject (No. 32), the specific gravity was 1.020 but the maximal osmotic U/P ratio was only 1.51.

Column 15 indicates the per cent of concurrently filtered water which was excreted during maximal osmotic diuresis. The administration of mannitol was relatively constant in all patients and not adjusted to the filtration rate and consequently higher plasma concentrations were undoubtedly obtained in patients with low filtration rates. This circumstance would explain the fact that there is a roughly inverse correlation between the fraction of filtered water excreted at the peak of diureses and the filtration rate.

DISCUSSION

Our observations show that a renal mechanism for the production of an osmotically concentrated urine persists throughout the course of glomerulonephritis. The tendency for the ratio $T^c_{H_2O}/C_{IN}$ to be elevated early in the course of the disease suggests predominant glomerular damage. Maintenance of a normal ratio as the disease progresses suggests proportional glomerular and tubular impairment, probably in consequence of loss of function in entire nephrons. It is only in the most advanced stages of functional impairment that a markedly reduced $T^c_{H_2O}/C_{IN}$ ratio provides evidence of a specific tubular defect, but here the reduction in ratio may be attributable to a relative increase in filtration rate. Our data are therefore inadequate to establish whether a defect in concentrating power is characteristic of glomerulonephritis. Certainly in many patients with fairly advanced disease (filtration rates of 40 cc. per min. or less) residual concentrating power is proportional to the residual filtration rate.

With respect to acute renal failure, our data show that concentrating capacity is reduced out of proportion to the reduction in filtration rate.

Among the 25 subjects examined here, the slope of the regression line lies between 0.90 and 1.10 in 14, and between 1.10 and 1.30 in 9. We can add nothing to the remarks of Zak, Brun, and Smith on the obscure factor or factors which cause this slope to deviate from 1.0, except to re-emphasize that, in view of the high coefficient of correlation with linear regression, this deviation must be considered to be physiologically significant.

It must be noted that in the two subjects (Nos. 35 and 9), where the slope of the regression line is very low (0.877 and 0.824, respectively), the calculation of a maximal value of $T^c_{H_2O}$ is at present meaningless since $T^c_{H_2O}$ becomes negative at urine flows of 8.5 and 1.8 cc. per min., respectively; *i.e.*, both subjects excreted a hypotonic urine during osmotic diuresis in spite of sustained hydropenia and the administration of Pitressin®. Whatever the cause for the low slope, it operates consistently throughout the test, as shown by a high correlation coefficient (0.9905 and 0.9999). The explanation of this deviation in slope, with respect to the presumed constancy of $T^c_{H_2O}$ at adequate urine flows, must remain for further investigation. It is perhaps significant that in both of these subjects, a relatively large fraction (67 and 55 per cent) of the filtrate as measured basally was excreted during osmotic diuresis, and that in both, the filtration rate increased markedly during osmotic diuresis.

At urine flows where $T^c_{H_2O}$ has attained its maximal value, the osmotic U/P ratio decreases with increasing osmotic diuresis in accordance with the relationship (which issues from equation *1*):

$$(2) \qquad \frac{U_{osm}}{P_{osm}} = 1 + \frac{T^c_{H_2O}}{V}$$

and neither U_{osm} nor U_{osm}/P_{osm} affords any direct information on the concentrating operation of the kidney, which is properly judged by $Tm^c_{H_2O}$ itself.

At progressively lower urine flows, as noted above, water reabsorption proceeds until the osmotic pressure of the urine, or the osmotic U/P ratio, approaches a maximal value.

It is under conditions of oliguria, where this limitation applies, that the maximal urine specific gravity is generally measured. Discrepancies can be expected between urine specific gravity and osmotic concentration since the contribution of the several urinary constituents to specific gravity and osmotic concentration are quite different.[7]

Although our studies were not designed to examine the maximal osmotic U/P ratio *per se*, none of our subjects failed to elaborate urine of higher osmotic concentration than plasma, in spite of the fact that the specific gravity of the same short-period samples collected after 18 hours of fluid deprivation was 1.013 or less in 8 of the 16 instances in which the comparison was made. Three patients (Nos. 19, 37, 34) in the early stages of glomerulonephritis ($C_{IN} > 60$ cc. per min.) showed a maximal specific gravity of 1.013 to 1.015, though the concentrating mechanism, as indicated by $T^c_{H_2O}/C_{IN}$ ratios of 8.8, 6.6, and 5.3, was well preserved. These subjects also showed good maximal osmotic U/P ratios (3.28, 2.30, 2.50). It is clear that specific gravity is only an approximate index of osmotic concentration.

Data are not yet available on the relation of $Tm^c_{H_2O}$, as measured during osmotic diuresis, and the maximal osmotic U/P ratio during oliguria. It seems improbable that there exist two mechanisms for the reabsorption of solute-free water, one operating in oliguria and the second during osmotic diuresis. If it is accepted that the concentrating process represents a unitary mechanism operating at all urine flows, then when $Tm^c_{H_2O}$, as measured during osmotic diuresis, is zero, the urine during oliguria must be isosmotic with the plasma, *i.e.*, the osmotic U/P ratio will be 1.0, regardless of the urine flow. Conversely, where the oliguric osmotic U/P ratio is 1.0, it is not to be expected that $Tm^c_{H_2O}$ will have a positive value at any level of osmotic diuresis. But in as much as the maximal osmotic U/P ratio may represent a limiting osmotic gradient across the renal tubules or collecting ducts (or less immediately, the ureters and bladder), it is conceivable that this ratio may be decreased in disease when $Tm^c_{H_2O}$ still retains a substantial value.

The difficulty in examining this question, and of quantitatively assessing the maximal osmotic U/P ratio, is that in oliguria the kidney is in a mild but highly variable state of "osmotic diuresis" attributable to the continued excretion of urea, creatinine, sodium chloride, *etc.* (9). Insofar as there is substantial splay at the intermediate urine flows where we pass from the parameter of maximal osmotic U/P ratio to the parameter of $Tm^c_{H_2O}$, the observed osmotic U/P ratio may be significantly depressed by this "endogenous" osmotic diuresis. Our present values for the maximal osmotic U/P ratio must therefore be looked upon as possibly less than "maximal." The measurement of the maximal U/P ratio must await detailed studies of the experimental conditions under which it can be attained, in the normal as well as in the diseased kidney.

CONCLUSION

1. The urinary concentrating operation during osmotic diuresis and Pitressin® administration has been examined in 25 hydropenic subjects with impaired renal function: 19 with acute or chronic glomerulonephritis, 1 with chronic pyelonephritis, 2 with Kimmelstiel-Wilson syndrome, and 3 with acute renal failure.

2. The quantity of solute-free water ($Tm^c_{H_2O}$) required to restore the urine to an isosmotic state with respect to the glomerular filtrate was calculated as the difference between osmolar clearance and urine flow.

3. A concentrating operation of substantial magnitude was demonstrated in all subjects with glomerulonephritis. In five subjects with acute glomerulonephritis, who in all instances had a filtration rate (C_{IN}) above 60 cc. per min., the ratio of $Tm^c_{H_2O}$ ranged from 5.3 to 8.8, and thus exceeded the mean normal value of 5.1. We interpret these data as indicating that early in glomerulonephritis the filtration rate may be reduced without substantial impairment of the concentrating operation represented by $Tm^c_{H_2O}$. Conversely, in 10 subjects with chronic glomerulonephritis in whom the filtration rate was below 60 cc. per min., the ratio $Tm^c_{H_2O}$ was above 5.0

[7] We find no warrant for the rigid correlation between specific gravity and osmotic pressure implied by Figure 4 of Darrow and Pratt (8).

in five; in the rest, this ratio ranged from 3.0 down to 1.5. Our data are too limited to determine whether in advanced glomerulonephritis the concentrating operation may be consistently impaired out of proportion to impairment in filtration rate.

4. In the three subjects examined after an episode of acute renal failure, the ratio $Tm^{c}_{H_2O}/C_{IN}$ was so low that we infer the presence of specific damage to the concentrating mechanism.

5. The maximal specific gravity of urine collected after an 18-hour period of hydropenia does not in general correlate with either the magnitude of the concentrating operation as measured by $Tm^{c}_{H_2O}$, or with the osmotic U/P ratio determined in these same samples. At a specific gravity of 1.013 or lower, osmotic U/P ratios as high as 2.5 may be reached. This lack of correlation is clearly attributable to the fact that different solutes (sodium chloride, urea, *etc.*) contribute differently to osmotic pressure and to specific gravity.

REFERENCES

1. Wesson, L. G., Jr., and Anslow, W. P., Jr., Effect of osmotic and mercurial diuresis on simultaneous water diuresis. Am. J. Physiol., 1952, **170**, 255.
2. Zak, G. A., Brun, C., and Smith, H. W., The mechanism of formation of osmotically concentrated urine during the antidiuretic state. J. Clin. Invest., 1954, **33**, 1064.
3. Smith, H. W., The Kidney: Structure and Function in Health and Disease. New York, Oxford University Press, 1951.
4. Schreiner, G. E., Determination of inulin by means of resorcinol. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 117.
5. Bowman, R. L., Trantham, H. V., and Caulfield, P. A., An instrument and method for rapid, dependable determination of freezing-point depression. J. Lab. & Clin. Med., 1954, **43**, 310.
6. Johlin, J. M., The freezing point determination of physiological solutions. The usual errors and their elimination. J. Biol. Chem., 1931, **91**, 551.
7. Smith, H. W., Renal excretion of sodium and water. Federation Proc., 1952, **11**, 701.
8. Darrow, D. C., and Pratt, E. L., Fluid therapy. Relation to tissue composition and the expenditure of water and electrolyte. J. A. M. A., 1950, **143**, 365.
9. Platt, R., Renal failure. Lancet, 1951, **1**, 1239.

SIMULTANEOUS ESTIMATION OF RATES OF THYROXINE DEGRADATION AND THYROID HORMONE SYNTHESIS[1]

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Implicit in many studies of thyroidal function is the assumption that the rates of synthesis and degradation of thyroid hormone are equal (1–4). Yet this assumption has not been critically examined. Constancy of concentrations of serum precipitable iodine (SPI), particularly under conditions of environmental stress, need not indicate an equilibrium of this type, for interposed between the site of hormonal synthesis and the peripheral circulation is a large pool of stored hormone (1, 4–6). This pool is capable of maintaining, at least for a time, constancy of SPI, despite marked discrepancies between rates of hormonal production and degradation (7).

A method has been devised for simultaneous estimation of the rate of degradation of thyroxine within the body and the rate of iodination of organic moieties within the thyroid gland.[4] The equivalence of these two functions has been assessed in patients with diverse thyroidal states. In addition, the method has been applied to an evaluation of several factors which influence the degradation of thyroxine.

MATERIAL AND ANALYTIC METHODS

The group studied included nine euthyroid individuals, five patients with untreated myxedema, nine with treated myxedema, five with Graves' disease, and three with panhypopituitarism. Diagnoses were established on the basis of clinical picture, determination of BMR, thyroidal uptake of I^{131}, SPI, and when necessary, measurements of adrenal and pituitary gonadotrophic function. All patients were studied on a metabolic ward, receiving constant weighed diets ranging from 2,000 to 4,000 in caloric content.

Each patient received from 3 to 5 μg. of l-thyroxine intravenously,[5] labelled with 30 to 50 μc of I^{131}. At 24-hour intervals the thyroidal content of radioiodine was measured with a directional scintillation counter (sodium iodide crystal, thallium activated) positioned 20 on from the neck. Radioactivity over the midthigh was subtracted from that observed over the neck in order to "correct" for extrathyroidal radioactivity. Samples of serum and 24-hour collections of urine were obtained daily in carefully cleaned glassware. In patients with thyrotoxicosis, samples of serum were obtained twice daily. In patients in whom fecal collections were made, powdered carmine was used as a marker. Weighed aliquots of homogenized stool were prepared. Radioactivity in all samples of serum, urine, and feces was determined in a well-type scintillation counter (sodium iodide crystal, thallium activated), and in all instances was expressed as a percentage of the administered dose. Corrections for physical decay were obviated by comparing the activity of all samples to that of a suitably diluted fraction of the administered dose. Sufficient counts were observed to reduce the probable error of results to less than one per cent. Analyses for stable iodine were performed by the method of Barker, Humphrey, and Soley in serum (8) and the method of Barker in urine (9).[6] Statistical analyses were performed according to methods described by Snedecor (10).

METHOD OF ANALYSIS

Following the intravenous administration of radioactive thyroxine (Tx^*), the virtual volume of distribution of injected material was calculated daily according to the principle employed by Myant and Pochin and by Benua, Albert, and Keating in the study of the distribution of radiothyroxine in man (11, 12).
Thus, where,

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[2] Howard R. Hughes Fellow in Medicine.

[3] Fellow of the American Cancer Society, Inc., recommended by the Committee on Growth of the National Research Council.

[4] In the ensuing discussion, iodinated organic moieties stored within the thyroid gland, regardless of their specific chemical nature, will be termed "thyroid hormone."

[5] Obtained from Abbott Laboratories, Chicago, Ill.

[6] Performed by Bioscience Laboratories, 2231 Carmelina Avenue, Los Angeles 64, Calif.

TDS = The "thyroxine distribution space" or volume of distribution of thyroxine at any time, t (liters).

R = Residual radiothyroxine (Per cent of the administered radiothyroxine remaining within the thyroxine space at any time, t. Total dose minus sum of thyroidal, fecal and urinary collections of I^{131} up to time, t).

P = Concentration of Tx* in plasma at time, t (per cent dose per liter).

$$TDS = R/P.$$

Mixing of Tx* was assumed to be complete when calculated values of TDS became constant. The subsequent slope of the exponential curve depicting the concentration of Tx* in the plasma was considered to represent solely the metabolism of labelled hormone. The time required for the concentration of isotope to decrease by 50 per cent (half-time, $t_{1/2}$) was determined by inspection of the metabolic slope, and the fractional rate of turnover of thyroxine (k) was calculated according to the formula,

$$k \text{ (proportion/day)} = 0.693/t_{1/2} \text{ (days)}.$$

In addition, where

V = Daily volume turnover or the volume of the TDS whose hormone is replaced daily (liters per day).

ETT = Extrathyroidal thyroxine. Thyroxine contained within the TDS (μg. thyroxine I).

SPI = Serum precipitable iodine (μg. I per 100 ml.).

D = Thyroxine degradation rate (μg. thyroxine I per day).

$$V = TDS \times k$$
$$ETT = TDS \times SPI \times 10$$
$$D = V \times SPI \times 10.$$

During the degradation of radioactive thyroxine, radioiodine accumulates in three sites: the thyroid, urine, and feces (Figure 1). That within the urine and thyroid has been demonstrated to arise principally from inorganic I^{131} liberated by the peripheral degradation of thyroid hormone (11–13). Fecal radioactivity has been shown to be largely organic (13). The plasma's radioactivity was considered to consist entirely of Tx*. However, since inorganic radioiodine must certainly have entered thyroid and urine from the plasma, a fraction of the plasma's radioiodine must at all times have been inorganic. Previous authors have considered this fraction to be negligibly small (11–13). One estimate of the magnitude of this inorganic moiety could be derived by substituting in the conventional clearance formula the daily excretion of radioiodide and an assumed daily renal iodide clearance of 48 liters (11). Inorganic radioactivity in plasma was found by this technique to contribute no more than 1 to 2 per cent to the total radioactivity of plasma. Furthermore, in 11 patients, 93 to 99

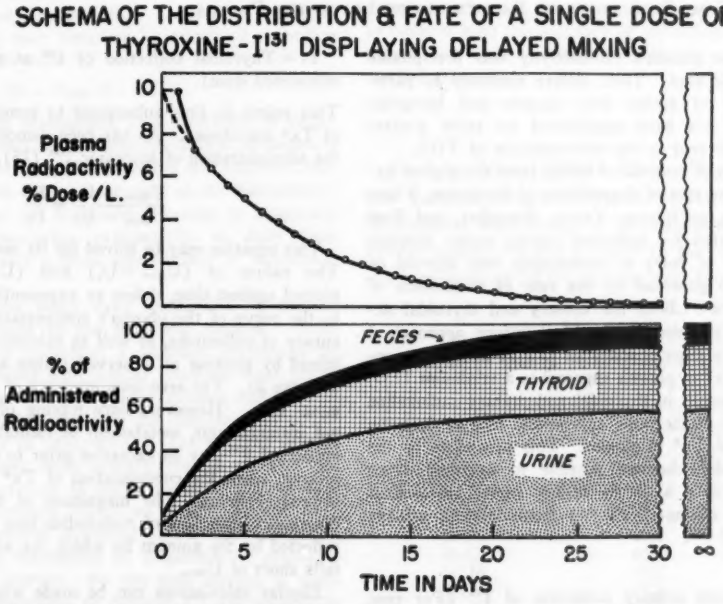

FIG. 1. FATE OF A SINGLE INTRAVENOUS DOSE OF RADIOACTIVE THYROXINE

Fecal, urinary, and thyroidal accumulation of I^{131} approach their maximum at the same exponential rate at which the concentration of radiothyroxine in plasma approaches a minimum. Metabolism of radiothyroxine prior to completion of mixing results in an early rapid accumulation of I^{131} in urine, thyroid, and feces.

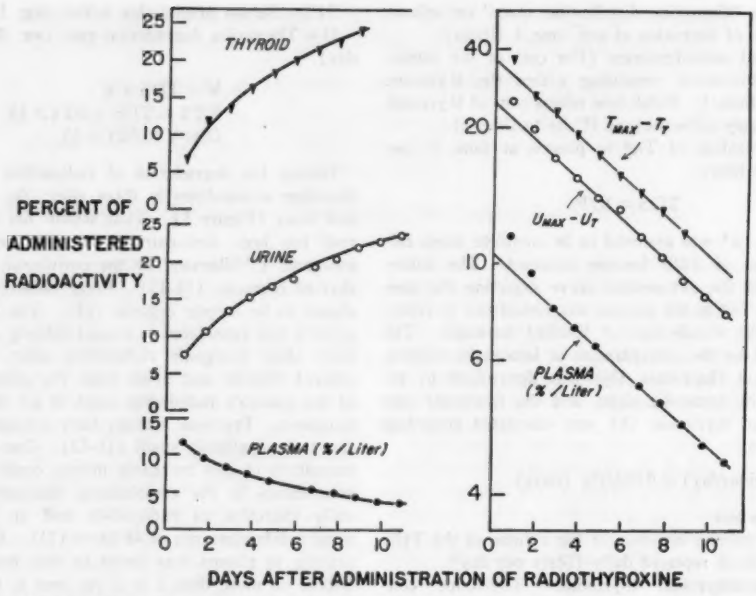

FIG. 2. FATE OF INTRAVENOUSLY ADMINISTERED RADIOTHYROXINE IN A EUTHYROID PATIENT, DEMONSTRATING THAT RADIOIODINE ENTERS THE THYROID AND URINE AT THE SAME RATE AT WHICH RADIOTHYROXINE LEAVES THE PLASMA

per cent of the plasma's radioactivity was precipitable by trichloracetic acid. Thus, failure routinely to partition specimens of serum into organic and inorganic moieties could not have contributed an error greater than several per cent in the determination of TDS.

Since the rate of removal of iodide from the plasma exceeds greatly the rate of degradation of thyroxine, it was anticipated that, as Berson, Yalow, Schreiber, and Post have demonstrated for iodinated human serum albumin (14), the rate of entry of radioiodide into thyroid or urine would be governed by the rate of breakdown of administered Tx*. Both the urinary and thyroidal accumulation of radioiodine should therefore approach a maximum at the same exponential rate at which the concentration of Tx* in plasma approaches a minimum (15). The daily increment in thyroidal and urinary radioiodine should diminish at the same exponential rate as does the concentration of Tx* in plasma. This proved to be the case. It therefore became possible to calculate, from data collected over a 10 to 16-day period, theoretical values for the ultimate ($t = \infty$) thyroidal and urinary accumulation of I^{131}.

If

U_{Max} = Ultimate urinary collection of I^{131} (Per cent administered dose).

T_{Max} = Ultimate thyroidal collection of I^{131} (Per cent administered dose).

U_t = Urinary collection of I^{131} at any time, T (Per cent administered dose).

T_t = Thyroidal collection of I^{131} at any time, t (administered dose).

Two points in time subsequent to completion of mixing of Tx* are chosen. As has been demonstrated following the administration of inorganic I^{131} (15).

$$\frac{U_{Max} - U_1}{U_{Max} - U_2} = \frac{P_1}{P_2}$$

This equation may be solved for its one unknown, U_{Max}. The values of $(U_{Max} - U_1)$ and $(U_{Max} - U_2)$, when plotted against time, define an exponential curve, parallel to the curve of the plasma's concentration of Tx*. Accuracy of collections, as well as calculation can be ascertained by plotting all observed values against this curve (Figure 2). The zero-time intercept of this curve should equal U_{Max}. However, since mixing of injected Tx* is not instantaneous, metabolism of radiothyroxine and excretion of I^{131} may be excessive prior to the completion of mixing, when the concentration of Tx* in plasma is inordinately great. The magnitude of this excessive or disproportionate loss of radioiodide into the urine will be reflected by the amount by which the zero-time intercept falls short of U_{Max}.

Similar calculations can be made with values of thyroidal I^{131}. Since the total dose of radioiodine eventually appears in thyroid, urine, or feces, it then becomes possible to calculate by the following formula a theoretical maximum fecal collection of I^{131} (F_{Max}).

$$F_{Max} = 100 - (U_{Max} + T_{Max}).$$

TABLE I

Correlation between predicted and observed values of fecal excretion of I^{131}

Pt.	Interval†	Fecal I^{131} (% dose) Predicted	Observed
R. F.*	0–5	9.6	12.0
	5–10	5.9	5.8
M. F.*	0–5	8.1	7.4
S. F.	0–5	3.9	4.5
E. G.	0–5	5.5	5.4
M. McC.	0–5	11.3	10.4
	5–10	6.5	5.1
L. McK.*	0–5	3.9	3.9
	5–10	2.3	1.2
V. M.*	0–5	2.1	3.3

* Values obtained while patient receiving cortisone. Complete results will be presented in a subsequent publication.

† Days after administration of radiothyroxine.

It may be assumed that fecal collection of I^{131} proceeds at the same exponential rate as loss of Tx* from the plasma. This assumption is borne out by the data of Benua, Albert, and Keating (12). Cumulative fecal loss at any time (F_t) may therefore be calculated according to the following formula.

$$F_t = F_{Max}\,(1 - e^{-kt}).$$

The accuracy of determining fecal excretion of radioiodine in this indirect manner was checked in 10 instances (Table I). In no case did calculated and observed values differ by more than 2.4 per cent of the administered dose. Because of the difficulties inherent in accurately collecting and measuring radioactivity in daily fecal samples, the indirect method was utilized in subsequent patients.

Estimates of the mean daily production of thyroid hormone were made in the following manner. From each daily urinary collection, a standard percentage (1 per cent) was pooled with similar fractions from previous days. Analysis of the pooled urinary samples revealed the geometrical mean of the concentration of I^{127} during the period of observation. The product of this value and the mean daily urinary volume revealed the average daily excretion of stable iodine. The increase in thyroidal and urinary collections of I^{131} (ΔT and ΔU) between the completion of mixing of Tx* and completion of the study was determined by inspection of appropriate accumulation curves. By the time mixing is complete negligible quantities of organic I^{131} are present in the urine (11). It has further been demonstrated that following the administration of Tx*, the relative rates of entry of radioiodine into thyroid and urine are the same as those which follow the administration of inorganic I^{131}. These in turn may be considered to represent the relative rates of entry of inorganic I^{127} into these two sites (11, 12). Therefore,

$$\frac{\Delta U}{\Delta T} = \frac{\text{daily urinary excretion of } I^{127}}{\text{daily thyroidal accumulation of } I^{127}}.$$

This equation is then solved for its one unkown, the daily thyroidal accumulation of I^{127}, or rate of production of hormone.

Observation of the urinary and thyroidal accumulation of I^{131} following the administration of radiothyroxine also makes possible calculation of the fraction of liberated radioiodide which enters the thyroid gland. This has been designated the thyroidal fraction (T.F.).

$$\text{T.F. (per cent)} = \frac{\Delta T \times 100}{\Delta T + \Delta U}.$$

Since radioiodide liberated by the peripheral degradation of Tx* apportions itself between thyroid and urine as does a tracer dose of inorganic radioiodide, the thyroidal fraction should be numerically equal to the ultimate thyroidal uptake of a tracer dose of inorganic I^{131}. In 10 patients, values of the thyroidal fraction were compared with the uptake observed 48 hours after a usual tracer dose of radioiodide. In no instance did the thyroidal fraction differ from the observed uptake by more than 5.3 per cent of the administered dose; the mean difference between the two being 0.2 per cent uptake (Table II). The close agreement noted between predicted and observed values of the radioiodide uptake would appear to confirm both the accuracy of collections and measurements as well as the basic assumptions upon which the method depends.

RESULTS

Euthyroidism: (Table III.) The SPI in normal patients ranged from 3.0 to 7.0 μg. per cent. Thyroxine was found to be distributed in an average volume of 9.4 L. and to be replaced at an average rate of 10.6 per cent per day. Mean vol-

TABLE II

Correlation between predicted and observed values of thyroidal fraction

Patient	Thyroidal fraction (per cent) Predicted	Observed
M. B.	29.5	24.2
H. F.	35.1	32.4
B. H.	29.0	30.0
B. G.	44.8	46.3
V. M.	40.1	43.5
D. N.	21.7	21.4
R. N.	22.7	21.7
R. R.	26.0	29.9
R. R.	22.1	24.5
R. W.	40.2	35.4
Mean	31.1	30.9

TABLE III

*Summary of values obtained in diverse states of thyroidal function**

Pt.	Sex	TDS (liters)	k (%/day)	V (ml./day)	SPI (μg. I %)	ETT μg. I	D (μg. I/day)	F_{max} (% dose)	TF (%)	Urinary iodine (μg./day)	Hormone product. (μg. I/day)
Myxedema, untreated											
P. C.†	M	7.9	9.77	772	1.1	87	8.5	23.0	0		0
E. F.	F	8.0	8.25	660	1.2	96	7.9	11.6	0		0
F. G.	F	7.4	10.34	765	1.3	96	9.9	28.2	0		0
V. M.	F	6.6	11.74	763	2.5	165	19.1	22.5	40.1	79.3	53.1
L. McK.†	F	7.5	8.89	667	1.2	90	8.0	18.9	0		0
Mean		7.5	9.80	725	1.5	107	10.7	20.8			
Std. Dev.		0.6	1.36	57	0.6	33	4.8	7.1			
Myxedema, treated											
P. C.	M	7.9	10.19	805	4.8	379	38.6	25.9	0		0
M. F.	F	9.8	9.76	956	6.3	617	60.2	24.3	0		0
E. G.	F	11.7	10.66	1,247	3.2	375	39.9	13.0	0		0
V. M.	F	7.0	10.83	758	4.7	329	35.6	17.9	20.6	145.5	37.8
M. McC.	F	8.1	10.83	878	9.0	729	79.0	27.0	0		0
L. McK.	F	7.0	9.76	683	6.0	420	41.0	20.0	0		0
R. R.	M	9.4	8.35	785	3.4	320	26.7	24.7	0	246.0	0
G. S.	F	9.5	9.00	855	4.4	418	37.6	18.1	0		0
A. W.	F	9.2	11.74	1,080	6.4	589	69.2	15.9	0		0
Mean		8.8	10.12	894	5.4	464	47.5	20.8			
Std. Dev.		1.4	1.03	170	1.8	145	17.6	4.9			
Normal thyroidal function											
A. B.	M	9.1	8.88	808	5.1	464	41.2	26.3	37.1	144.6	85.4
R. C.	M	10.4	11.00	1,144	6.0	624	68.7	33.2	39.8	107.2	70.8
H. F.	F	6.5	10.50	683	7.0	455	47.8	10.3	35.1	96.9	52.4
B. G.	F	7.7	10.66	821	4.4	339	36.1	33.8	44.8	50.9	41.3
B. H.	F	10.1	9.49	958	7.0	707	67.1	28.9	29.0	155.2	63.4
D. M.	M	9.5	11.36	1,079	3.0	285	32.4	15.2	20.4	130.5	33.4
D. N.	F	10.6	10.83	1,148	5.5	583	63.2	36.4	21.7	203.3	56.4
R. N.	M	8.0	10.34	827	5.6	448	46.3	36.1	22.7	142.5	41.9
D. P.	M	12.8	11.95	1,532	5.2	666	79.6	23.0	31.1	168.6	76.0
Mean		9.4	10.56	1,000	5.4	508	53.6	26.9	31.3	133.3	57.9
Std. Dev.		2.0	0.93	258	1.3	146	16.5	9.2	8.6	44.2	17.5
Hyperthyroidism											
A. B.	F	8.1	21.66	1,754	17.0	1,377	297	18.0	91.6	101.0	1,101
A. L.	F	10.8	18.73	2,022	17.5	1,890	354	22.4	84.0	211.8	957
D. L.	M	4.7	31.50	1,480	14.6	686	216	35.9	81.4	121.0	530
S. W.	F	12.8	25.67	3,260	17.4	2,222	567	25.0	78.0	273.3	970
Mean		9.1	24.39	2,129	16.6	1,544	359	25.3	83.8	176.8	890
Std. Dev.		3.5	5.53	770	1.4	670	150	7.6	5.8	80.4	248
M. T.‡	M	6.9	16.12	1,112	9.0	621	100	16.8	0		0
Panhypopituitarism											
M. B.	F	11.6	7.14	828	3.0	348	24.7	31.2	29.5	71.2	29.8
E. D.	F	7.5	8.66	650	2.6	195	16.9	17.8	16.0	110.0	21.0
R. R.†	M	8.6	7.53	648	1.9	163	12.3	19.4	26.0	43.3	16.1
Mean		9.2	7.78	709	2.5	235	18.0	22.8	20.5	74.8	22.3

* See text for explanation of symbols.

† Studied again after therapy with desiccated thyroid.

‡ Patient received 300 mg. propylthiouracil and 30 qts. Lugol's solution daily during study. SPI is pre-treatment value.

ume turnover was 1000 ml. per day. The normal extrathyroidal content of thyroxine averaged 508 μg. hormonal iodine and 53.6 μg. of hormonal iodine were utilized per day.

Treated myxedema: All patients were judged to be euthyroid on the basis of clinical picture and BMR. SPI in this group ranged between 3.2 and 9.0 μg. per cent. Values for all functions agreed well with those of the euthyroid group.

Primary myxedema: In the five subjects comprising this category, SPI ranged from 1.1 to 2.5 μg. per cent. A significant reduction of the TDS was observed (mean, 7.5 L.; $p < .02$). Although the fractional rate of turnover of thyroxine was somewhat less than normal, a statistically significant reduction could not be demonstrated. As a result of the diminution in these functions, the average daily volume turnover of the thyroxine space was significantly reduced (mean, 725 ml.; $p < .01$). Similarly total extrathyroidal thyroxine (mean, 10.7 μg. I; $p < .01$) and the daily rate of utilization of thyroxine (mean, 10.7 μg I per day; $p < .01$) were significantly less than normal.

Panhypopituitarism: Because of the small number of patients available for study, statistical analysis of results in this group was not carried out. The distribution and rate of turnover of thyroxine appeared to be similar to that observed in patients with untreated myxedema, but SPI was uniformly greater, and consequently, utilization of hormone, although subnormal, was considerably more than that encountered in the myxedematous group.

Hyperthyroidism: Studies were performed in four patients with untreated hyperthyroidism whose SPI's ranged from 14.6 to 17.5 μg. per cent. For the following reasons, other results in this group must be considered approximations of those which truly pertain. Radioiodine liberated by the peripheral degradation of thyroxine is apportioned between the thyroid and the urine. In patients with thyrotoxicosis, the larger fraction of this inorganic radioiodide enters the thyroid gland. Once incorporated into hormone, the radioiodine is rapidly released into the circulation (1, 16). The accelerated turnover of radioiodine in the thyrotoxic gland results in a progressive underestimation of the thyroidal accumulation of I^{131} and in a restoration to the thyroxine space of a portion of the radiothyroxine initially injected. Thus, there results an overestimation of the plasma's concentration of exogenously administered Tx* and an underestimation of the rapidity of its degradation. Finally, as the endogenously synthesized radiothyroxine is degraded, a fraction of the radioiodide thereby liberated will also enter the urine and hence will augment the urinary radioactivity resulting from the initial cycling of Tx*.

That this sequence of events did transpire in patients with untreated thyrotoxicosis is suggested by the following observations. In contrast to the findings of patients in other clinical categories, in whom a continuously increasing thyroidal content of I^{131} was noted, thyroidal radioactivity increased during the first several days after administration of Tx*, and then either decreased or remained constant. Similar curves of the thyroidal accumulation of I^{131} following the administration of Tx* to hyperthyroid patients have been noted by Benua, Albert, and Keating (12). When the daily increment in thyroidal I^{131} during the first several days was plotted, an exponential curve with a slope parallel to that of the plasma's concentration of Tx* was defined. Subsequent points, however, fell progressively below a projection of the initial slope.

It is well known that radioactive hormone may be released from the thyroid gland within a few hours after the initial penetration of inorganic I^{131} (17). However, in the present system, the earliest gross manifestation of hormonal release was the appearance of a deficit in the thyroidal increment of radioiodine, as noted above. This was mirrored in the urine by an excessively large daily increment in I^{131} and in the plasma by a retardation of the rate of disappearance of radiothyroxine.

If endogenously synthesized radiothyroxine were instantaneously mixed throughout its distribution compartment, these events would not alter values of TDS obtained by the present method, which relates the amount of Tx* within the thyroxine space to its concentration therein. Only the observed fractional rate of turnover would be affected (decreased). However, mixing of thyroxine is not instantaneous. As a consequence, the magnitude and direction of errors in the estimation of the thyroxine space will depend upon two factors: a) The relative rapidities of hormonal turnover within the gland and hormonal mixing within the thyroxine space, and b) alterations in fecal losses resulting from retardation of

the net disappearance of thyroxine from the plasma.

Despite the foregoing difficulties, an attempt was made to derive approximate values in hyperthyroid patients by the method described. Calculations were based on data obtained only during the interval between the completion of mixing of administered radiothyroxine (*i.e.*, subsequent to 24 hours) and the first gross manifestation of release of radioactive hormone from the thyroid gland (72 to 120 hours).

The mean TDS of hyperthyroid patients did not appear to differ significantly from the normal value. The fractional rate of turnover was, however, markedly augmented (mean, 24.39 per cent per day; $p < .01$) as was daily volume turnover (mean 2,129 ml. per day ; $p < .02$). SPI ranged from 14.6 to 17.5 μg. per cent. Total extrathyroidal thyroxine (mean, 1544 μg. I; $p < .05$) and daily rate of utilization of thyroxine (mean, 359 μg. I; $p < .01$) were significantly greater than normal.

Since only early data were used in these calculations, it remained possible that the apparently rapid turnover rate might in part represent continued mixing of injected Tx*. Therefore, one untreated patient (M. T.) with thyrotoxicosis was given 300 mg. of propylthiouracil and 30 drops of Lugol's solution daily during the determination of his rate of degradation of thyroxine.[7] This medication completely inhibited the thyroidal accumulation of radioiodine, and thereby prevented the recycling of radiothyroxine. Despite the fact that the patient experienced rapid and nearly complete symptomatic response during the period of observation, the fractional rate of turnover of thyroxine was more rapid than that noted in any non-hyperthyroid patient, and fell near the range noted in other hyperthyroid patients who received no antithyroid therapy.

Production of thyroid hormone: Daily incorporation of I^{127} into the thyroid gland averaged 57.9 μg. iodine in euthyroid subjects and 890 μg. iodine in patients with untreated thyrotoxicosis. Patients with pituitary myxedema displayed a rate of hormonal production averaging 22.3 μg. iodine per day. Except in the case of patient V. M., thyroidal accumulation of radioiodine and consequently measurable thyroidal production of hormone could not be demonstrated in patients with primary myxedema. Patient V. M. has for years displayed classical signs and symptoms of hypothyroidism, together with abnormally low BMR and SPI, despite 24-hour thyroidal uptakes of I^{131} ranging from 40 to 50 per cent. The present studies revealed a normal rate of production of hormone, an abnormally low rate of degradation of thyroxine, and a diminished SPI. Preliminary studies in this patient suggest that her thyroid gland is producing and releasing into the circulation both thyroxine and a calorigenically less potent iodinated derivative whose solubility characteristics differ from those of thyroxine.

Relation between production of thyroid hormone and degradation of thyroxine: In individual patients with normal thyroidal function or panhypopituitarism, concomitantly measured rates of production of thyroid hormone and degradation of thyroxine were closely correlated ($r = 0.84 \pm 0.19$, $p = .002$). Analysis by the "paired t" test revealed no significant difference between paired values in individual patients ($p > 0.2$). In only one patient, A. B., a 45-year old alcoholic man with slowly resolving pneumonia, did paired values differ markedly. Although several patients in the normal group had recently been ill, A. B. was the only patient who had not convalesced completely at the time of study.

In thyrotoxic patients, calculated values for the daily production of hormone averaged 890 μg. and consistently exceeded estimates of the daily degradation of thyroxine.

DISCUSSION

Disappearance of hormone may reflect either a) utilization specifically associated with its action on an end organ, or b) degradation which is not linked to the mechanism of hormonal action. Since no known method will differentiate between these possibilities with regard to thyroid hormone, solely the term degradation is used herein to denote overall disappearance of thyroxine from its compartment of distribution.

Several previous studies of the degradation of radioactive thyroxine have employed conventional extrapolation techniques (3, 18–20). Potential errors of this technique are well known, and may

[7] Preliminary studies, employing patients with primary myxedema, indicate that iodides do not alter the rate of utilization of radioactive thyroxine.

influence both the measured space and the turnover rate (14, 21). These errors arise as a result of disproportionate metabolism of the labelled metabolite during the mixing period, failure to observe the plasma's slope for a sufficiently long period, and nonhomogeneity of the injected material.

The present method seeks to avoid these errors. The virtual volume of distribution of thyroxine at any time may be calculated as the quotient of the Tx* remaining in the space and its concentration therein. Rapidly metabolized contaminants, once cleared from the space, do not therefore influence the results. Constancy of calculated volumes of distribution indicates that mixing has been completed, and that the fractional rate of degradation may then be determined from the subsequent slope of the curve depicting the concentration of Tx* in the plasma.

By means of the present technique, the mean normal rate of degradation of thyroxine was found to be 53.6 μg. thyroxine iodine per day in normal subjects and 47.5 μg. thyroxine iodine per day in euthyroid patients with treated myxedema. These values agree with estimates of the daily requirement for hormone which others have derived, using a variety of methods (4, 20, 22).

As might be anticipated, breakdown of thyroxine was considerably increased in thyrotoxic subjects and profoundly diminished in patients with untreated myxedema. Values intermediate between those obtained in the euthyroid and the myxedematous groups were derived in patients with panhypopituitarism.

An examination of the constituent functions reveals the sources of differences in degradation of thyroxine noted. In normal patients thyroxine distributed in a volume of approximately 9.4 L. Approximately 10 per cent of the thyroxine contained within the space was replaced by newly released hormone each day. The thyroxine contained within approximately 1,000 ml. of the space was renewed each day. In myxedematous patients, the volume of the TDS was significantly diminished, and the fractional rate of turnover of Tx* was slightly reduced. As noted above, results in patients with hyperthyroidism must be interpreted with some reservations. Nevertheless, the TDS did not appear to differ from normal in patients with this disease.

Daily volume turnover of thyroxine did not differ significantly among patients with normal thyroidal function, treated myxedema, and panhypopituitarism. In patients with untreated myxedema, this function was reduced by about 25 per cent. Nevertheless, the large differences between the rates of degradation among these non-thyrotoxic groups were largely conditioned by and proportional to differences in the SPI. In the thyrotoxic patients, degradation was not proportional solely to the SPI, since the fractional turnover rate and therefore the daily volume turnover were markedly augmented (Figure 3). Proportional utilization of thyroxine, together with moderate reduction in the daily volume turnover of myxedematous patients may explain the relative ease with which the SPI can be elevated by standard doses of desiccated thyroid when the SPI is low, compared with the relative difficulty encountered in augmenting SPI when its concentration is normal or increased (23). Furthermore, it may be surmised that the disproportionately rapid degradation of thyroxine in the hyperthyroid patient, resulting from an increased fractional rate of turnover, would tend to lower the SPI and to make this measurement a rather insensitive index of the quantity of hormone used. It may further be noted that the relationship between SPI and degradation of thyroxine depicted in Figure 3 closely approximates the relationship between SPI and hormonal production and degradation, described by Burrows and Ross (24) and by Berson and Yalow (4), respectively.

Several further inferences concerning the metabolism of thyroxine may be drawn from the foregoing data. When administered to rats, large doses of thyroxine labelled with I^{131} are more rapidly metabolized than are small doses (25, 26). An analogy may therefore be sought in the rapid disappearance of thyroxine from the plasma of thyrotoxic patients. The more rapid removal of thyroxine under these circumstances could be ascribed to detoxification, perhaps performed by the liver (26–28). Although the present method does not ascertain the ultimate metabolic fate of thyroxine, it does permit evaluation of the relative quantity of hormonal iodine which is reduced to inorganic iodine and that which is excreted in the organic form *via* the feces. Normally approximately 25 per cent of I^{131} administered as Tx*

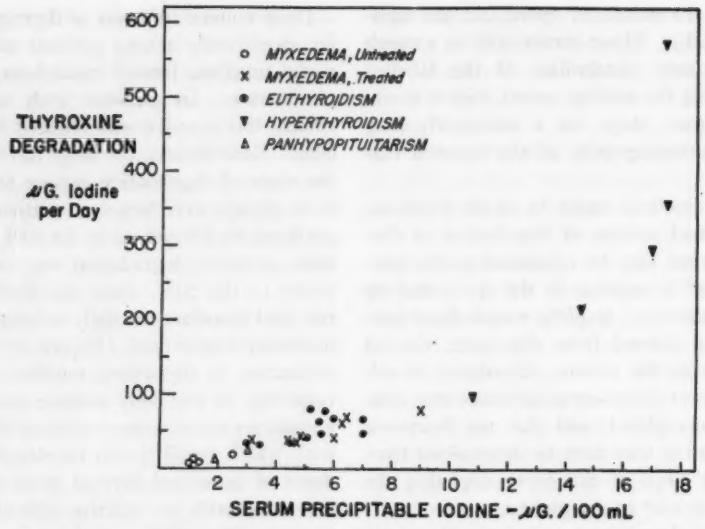

FIG. 3. RELATION BETWEEN THE CIRCULATING CONCENTRATION AND RATE OF DEGRADATION OF THYROID HORMONE

The relationship illustrated probably represents a continuous curved function. However, in the non-thyrotoxic range, the relationship approaches linearity.

appears in the feces, the remainder entering the thyroid or urine. In the hyperthyroid patient given radiothyroxine, this apportionment between fecal and non-fecal moieties did not differ significantly from the normal.

It has recently been suggested that augmented salivary deiodination of compounds containing organically bound iodine may occur in myxedema, and that salivary deiodination mechanisms, perhaps under hypophyseal control, may serve to maintain the balance of thyroid hormone in the body (29–31). There is ample evidence which the present study supplements, that thyroxine is the principle iodinated compound released by the thyroid gland. A predicated enhancement of deiodination in patients with myxedema is clearly not consistent with the observation that the fractional rate of turnover of thyroxine in such patients is, if anything, slower than that found in patients with normal thyroidal function or with treated myxedema.

The precise anatomical limits of the thyroxine space obviously cannot be defined. However, the values noted above for the distribution and rate of turnover of thyroxine are similar in magnitude to those previously reported for certain of the proteins in plasma (14, 32–34). In plasma, thyroxine is bound to an α-globulin (35–37). One may wonder whether the distribution and fate of thyroxine are related to the fate of the protein to which it is bound. The reduction in TDS in patients with untreated myxedema might there be analogous to the reduction in myxedema of the volume of dilution of dyes whose distribution is limited by their protein carrier (38). Similarly, alteration in the rate of turnover of thyroxine in patients with myxedema or thyrotoxicosis would then be consistent with parallel alterations in the rate of turnover of protein and other metabolites in these diseases (39–41). Studies designed to evaluate this hypothesis are in progress. However, preliminary observations suggest that the abnormally rapid removal of thyroxine seen in hyperthyroid patients may persist for months following the complete amelioration of the thyrotoxic state (7). The relationship of this metabolic abnormality to the inception or continuance of the disease state obviously remains to be clarified.

Although the present data do not make possible an appraisal of the metabolic fate and calorigenic importance of all iodinated compounds contained in thyroid extract, it was possible to demonstrate

TABLE IV

Relationship between degradation of thyroxine and dose of U.S.P. thyroid extract in patients with primary myxedema

Pt.	D.* μg. I/day	Thyroid dose gr./day	Ratio μg. I/gr.
G. S.	37.6	1.5	25.1
P. C.	38.6	2.0	19.3
E. G.	39.9	2.0	20.0
L. M.	41.0	2.0	20.5
M. F.	60.2	3.0	20.1
A. W.	69.2	3.5	19.8
			20.8±2.1

* Thyroxine degradation.

virtual equivalence between intake of exogenous thyroxine and degradation of this hormone. In six athyreotic patients under therapy with thyroid extract, an average of 21.0 μg. thyroxine iodine was degraded per grain of U.S.P. thyroid extract administered (Table IV). Since this preparation contains an average of 120 μg. total iodine, of which approximately 25 per cent is in the form of thyroxine (42), roughly 70 per cent of thyroxine apparently is absorbed. Of further interest was the observation that in the one myxedematous patient (V. M.) in whom measurable intrathyroidal iodinations were proceeding, daily administration of one grain of thyroid extract diminished this process by 15.3 μg. per day.

The present method does not necessitate inhibition of thyroidal accumulation of radioiodide liberated by the peripheral degradation of Tx*, and thus makes possible estimation of the relative rates of entry of inorganic iodide into thyroid and urine. Hence, measurements of the daily excretion of stable iodine may be used to calculate the daily thyroidal accumulation of I^{127}, or rate of production of hormone. In individual non-thyrotoxic subjects, close agreement has been noted between the daily incorporation of I^{127} into organic moieties and the concomitantly measured daily degradation of thyroxine (Figure 4).

Production and utilization of hormone in patients with panhypopituitarism although less than normal, exceeded considerably that found in patients with primary myxedema. The present data, which are apparently the first measurements of absolute rates of production of thyroid hormone in patients with pituitary myxedema, substantiate the lesser severity of myxedema arising from pituitary rather than from thyroidal failure.

In patients with hyperthyroidism, calculated values for the rate of production of thyroid hormone markedly exceeded values of the rate of degradation of thyroxine. This discrepancy could result from several factors. First, disparate values might represent a methodological artifact. Since, in thyrotoxic patients, the rapid turnover of iodine results in a diminution in the apparent thyroidal accumulation and an increase in the urinary excretion of I^{131}, it might be anticipated that rates of production of hormone would be underestimated. However, it is likely that rates of degradation are also underestimated. As noted earlier, release of radioactive hormone from the hyperfunctioning thyroid gland is rapid, and significant quantities of newly synthesized radiothyroxine may certainly have entered the circulation by the time mixing of exogenous Tx* was complete. It is likely that even the early slopes of disappearance of Tx* from plasma were slowed, and rates of degradation therefore underestimated. The relative magnitudes of these errors in the estimation of production and degradation cannot be assessed.

Alternatively, the observations may reflect a true disparity. Repletion of glandular stores of stable iodine during the period of observation (10 to 16 days) might be one of the means whereby thyroidal incorporation of iodine could exceed

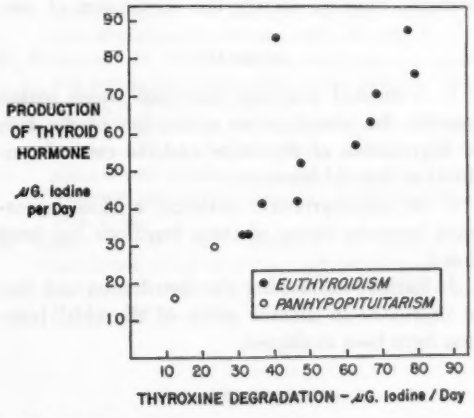

FIG. 4. RELATION BETWEEN RATES OF PRODUCTION OF THYROID HORMONE AND DEGRADATION OF THYROXINE IN NON-THYROTOXIC PATIENTS

the peripheral degradation of thyroxine. Similarly, release from the gland of quantities of thyroxine in excess of those being removed from the circulation could also be invoked. Although values of the SPI remained constant during the period of study, more prolonged observation might have revealed an increase in the concentration of circulating hormone. Another possible mechanism would be the synthesis and release of iodinated compounds other than thyroxine. Such compounds, *e.g.*, triiodothyronine, which are widely distributed and rapidly metabolized (18, 20), would be expected to contribute but little to circulating protein-bound iodine. Unfortunately, available data does not permit differentiation between these or other alternatives.

The present data demonstrate for the first time a close correlation between the rate of degradation of thyroxine and the rate of incorporation of iodine by the thyroid gland of the non-thyrotoxic patient. If, on the one hand, thyroxine is the principal iodinated compound produced and released by the thyroid gland, it may be concluded that mean glandular content of iodine remains constant over a period of 10 to 16 days. On the other hand, if it be assumed that glandular thyroxine remains constant, then thyroxine must constitute the principal end-product of thyroidal organic iodinations. One cannot logically conclude that either premise is necessarily true. However, their equivalence has been demonstrated by the present findings, and hence validation of both premises requires merely the verification of one.

SUMMARY

1. A method has been described which makes possible the simultaneous estimation of the rate of degradation of thyroxine and the rate of synthesis of thyroid hormone.

2. In non-thyrotoxic patients, a close agreement between values of these functions has been noted.

3. Factors influencing the distribution and fate of thyroxine in diverse states of thyroidal function have been evaluated.

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REFERENCES

1. Riggs, D. S., Quantitative aspects of iodine metabolism in man. Pharmacol. Rev., 1952, **4**, 284.
2. Ingbar, S. H., Kleeman, C. R., Quinn, M., and Bass, D. E., The effect of prolonged exposure to cold on thyroidal function in man. Clin. Res. Proc., 1954, **2**, 86.
3. Meszaros, J., and Robbins, R., Kinetics of protein-bound iodine release and utilization. Clin. Res. Proc., 1954, **2**, 85.
4. Berson, S. A., and Yalow, R. S., Quantitative aspects of iodine metabolism. The exchangeable organic iodine pool, and the rates of thyroidal secretion, peripheral degradation and fecal excretion of endogenously synthesized organically bound iodine. J. Clin. Invest., 1954, **33**, 1533.
5. Gutman, A. B., Benedict, E. M., Baxter, B., and Palmer, W. W., The effect of administration of iodine on the total iodine, inorganic iodine, and thyroxine content of the pathological thyroid gland. J. Biol. Chem., 1932, **97**, 303.
6. Ingbar, S. H., Freinkel, N., Hoeprich, P. D., and Athens, J. W., The concentration and significance of the butanol-extractable I^{131} of serum in patients with diverse states of thyroidal function. J. Clin. Invest., 1954, **33**, 388.
7. Ingbar, S. H., and Freinkel, N., Unpublished observations.
8. Barker, S. B., Humphrey, M. J., and Soley, M. H., The clinical determination of protein-bound iodine. J. Clin. Invest., 1951, **30**, 55.
9. Barker, S. B., Determination of protein-bound iodine. J. Biol. Chem., 1948, **173**, 715.
10. Snedecor, G. W., Statistical Methods Applied to Experiments in Agriculture and Biology. 4th ed., Ames, The Iowa State College Press, 1946.
11. Myant, N. B., and Pochin, E. E., The metabolism of radiothyroxine in man. Clin. Sc., 1950, **9**, 421.
12. Benua, R. S., Albert, A., and Keating, F. R., Jr., The metabolism of radiothyroxine in exophthalmic goiter. J. Clin. Endocrinol. & Metab., 1952, **12**, 1461.
13. Albert, A., and Keating, F. R., Jr., Metabolic studies with I^{131} labeled thyroid compounds. Comparison of the distribution and fate of radioactive d-l-thyroxine after oral and intravenous administration in the human. J. Clin. Endocrinol., 1949, **9**, 1406.
14. Berson, S. A., Yalow, R. S., Schreiber, S. S., and Post, J., Tracer experiments with I^{131} labeled human serum albumin: distribution and degradation studies. J. Clin. Invest., 1953, **32**, 746.
15. Ingbar, S. H., Simultaneous measurement of the iodide-concentrating and protein-binding capacity of the human thyroid gland. J. Clin. Endocrinol. & Metab., 1955, **15**, 238.
16. Freedberg, A. S., Chamovitz, D. L., and Kurland, G. S., Thyroid function in normal and pathological states as revealed by radioactive iodine studies.

I. Thyroid I^{131} uptake and turnover in euthyroid, hyperthyroid, and hypothyroid subjects. Metabolism, 1952, **1**, 26.

17. McConahey, W. M., Keating, F. R., Jr., and Power, M. H., The behavior of radioiodine in the blood. J. Clin. Invest., 1949, **28**, 191.
18. Rawson, R. W., Rall, J. E., Pearson, O. H., Robbins, J., Poppell, H. F., and West, C. D., L-triiodothyronine versus l-thyroxine, a comparison of their metabolic effects in human myxedema. Am. J. M. Sc., 1953, **226**, 405.
19. Hamolsky, M. W., Freedberg, A. S., Kurland, G. S., and Wolsky, L., The exchangeable thyroid hormonal pool. I. Its magnitude and rate of turnover in various thyroid states in man. J. Clin. Invest., 1953, **32**, 453.
20. Sterling, K., Lashof, J. C., and Man, E. B., Disappearance from serum of I^{131}-labeled l-thyroxine and l-triiodothyronine in euthyroid subjects. J. Clin. Invest., 1954, **33**, 1031.
21. Freinkel, N., Schreiner, G. E., and Athens, J. W., Simultaneous distribution of T-1824 and I^{131}-labelled human serum albumin in man. J. Clin. Invest., 1953, **32**, 138.
22. Stanbury, J. B., Brownell, G. L., Riggs, D. S., Perinetti, H., del Castillo, E., and Itoiz, J., The iodine-deficient human thyroid gland. A preliminary report. J. Clin. Endocrinol. & Metab., 1952, **12**, 191.
23. Greer, M. A., and Smith, G. E., Method for increasing the accuracy of the radioiodine uptake as a test for thyroid function by the use of desiccated thyroid. J. Clin. Endocrinol. & Metab., 1954, **14**, 1374.
24. Burrows, B. A., and Ross, J. F., The thyroidal uptake of stable iodine compared with the serum concentration of protein-bound iodine in normal subjects and in patients with thyroid disease. J. Clin. Endocrinol. & Metab., 1953, **13**, 1358.
25. Gross, J., and LeBlond, C. P., Metabolism of the thyroid hormone in the rat as shown by physiological doses of labeled thyroxine. J. Biol. Chem., 1950, **184**, 489.
26. Taurog, A., Briggs, F. N., and Chaikoff, I. L., I^{131}-labeled l-thyroxine. I. An unidentified excretion product in bile. J. Biol. Chem., 1951, **191**, 29.
27. Albert, A., and Keating, F. R., Jr., The role of the gastrointestinal tract, including the liver, in the metabolism of radiothyroxine. Endocrinology, 1952, **51**, 427.
28. Albert, A., Ford, E., and Lorenz, N., Effects of altered thyroid function upon the metabolism of endogenously labeled thyroid hormone. Endocrinology, 1953, **53**, 50.
29. Fawcett, D. M., and Kirkwood, S., Tyrosine iodinase. J. Biol. Chem., 1954, **209**, 249.
30. Thode, H. G., Jaimet, C. H., and Kirkwood, S., Studies and diagnostic tests of salivary-gland and thyroid-gland function with radioiodine. New England J. Med., 1954, **251**, 129.
31. Fawcett, D. M., and Kirkwood, S., Role of the salivary glands in extrathyroidal iodine metabolism. Science, 1954, **120**, 547.
32. Sterling, K., Serum albumin turnover in Laennec's cirrhosis as measured by I^{131}-tagged albumin. J. Clin. Invest., 1951, **30**, 1238.
33. Myant, N. B., Observations on the metabolism of human gamma globulin labelled by radioactive iodine. Clin. Sc., 1952, **11**, 191.
34. Vaughan, J. H., Armato, A., Goldthwaite, J., Brachman, P., Favour, C. B., and Bayles, T. B., Fate of intravenously injected labelled gamma globulin in the normal subject and in patients with rheumatoid arthritis. J. Clin. Invest., 1952, **31**, 668.
35. Gordon, A. H., Gross, J., O'Connor, D., and Pitt-Rivers, R., Nature of the circulating thyroid hormone-plasma protein complex. Nature, 1952, **169**, 19.
36. Deiss, W. P., Albright, E. C., and Larson, F. C., A study of the nature of the circulating thyroid hormone in euthyroid and hyperthyroid subjects by use of paper electrophoresis. J. Clin. Invest., 1952, **31**, 1000.
37. Robbins, J., and Rall, J. E., Zone electrophoresis in filter paper of serum I^{131} after radioiodide administration. Proc. Soc. Exper. Biol. & Med., 1952, **81**, 530.
38. Thompson, W. O., Studies in blood volume. I. The blood volume in myxedema, with a comparison of plasma volume changes in myxedema and cardiac edema. J. Clin. Invest., 1926, **2**, 477.
39. Rosenman, R. H., Byers, S. O., and Friedman, M., The mechanism responsible for altered blood cholesterol content in deranged thyroid states. J. Clin. Endocrinol. & Metab., 1952, **12**, 1287.
40. Hoberman, H. D., Endocrine regulation of amino acid and protein metabolism during fasting. Yale J. Biol. & Med., 1950, **22**, 341.
41. Crispell, K. R., Parson, W., and Hollifield, G. F., The amino acid pool and the protein synthesis rate in patients with primary myxedema before and after treatment with l-triiodothyronine. Clin. Res. Proc., 1954, **2**, 86.
42. Palmer, W. W., and Leland, J. P., Comparative calorigenic action of normal and pathological thyroid glands administered in equi-thyroxine doses. J. Clin. Invest., 1935, **14**, 619.

ETIOLOGIC RELATIONSHIP OF THE RI-67 AGENT TO "ACUTE RESPIRATORY DISEASE (ARD)"[1]

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As the result of intensive studies of acute respiratory infection in military populations from 1941 to 1946, an entity termed "acute respiratory disease" was described by the Commission on Acute Respiratory Diseases (1–4). This syndrome, for convenience, was termed "ARD," and this designation will be employed hereafter. ARD is an acute febrile respiratory infection of short duration with both constitutional symptoms and localized respiratory symptoms characterized predominately by cough and hoarseness (1, 2). In addition, irritated throat, nasal obstruction, and chest pain were frequent complaints. Epidemiological investigation (1) as well as human transmission experiments (3, 4) indicated that the incubation period of this infection was five to six days, and that during convalescence there developed an homologous immunity but not heterologous resistance to common cold or primary atypical pneumonia (4). It was postulated as a result of these investigations (1–4) that ARD was an entity distinguishable from other acute respiratory illnesses such as common cold, streptococcal pharyngitis and tonsillitis, and primary atypical pneumonia, and that it was caused by a single filterable agent, probably a virus, or a closely related group of agents. Attempts to isolate an agent in experimental animals and chick embryos were not successful.

Although ARD has not been recognized in epidemic form in civilian populations, similar infections such as non-streptococcal pharyngitis and grippe-like illnesses not caused by influenza viruses are frequently observed. Moreover, ARD continues to be an important respiratory infection in the Armed Forces. These considerations have stimulated continued investigation of this disease in order to delineate it further and to isolate the causative agent or agents.

The multiple isolations of cytopathogenic agents from tonsils and adenoids grown in tissue culture by Rowe, Huebner, Gilmore, Parrott, and Ward (5) indicated the existence of a new group of viruses which propagate in the upper respiratory tract of man and suggested a new approach to the investigation of acute undifferentiated respiratory infections. Independently, Hilleman and Werner (6) isolated in tissue culture 5 strains of cytopathogenic agents from cases of acute respiratory diseases which occurred during an influenza-like epidemic at Fort Leonard Wood, Missouri, during the winter of 1952–53. The majority of illnesses which occurred during this epidemic resembled ARD; some had pulmonary infiltration and were considered to be primary atypical pneumonia. Neither cold hemagglutinins nor agglutinins to streptococcus MG developed in patients with the latter type of illness. Hilleman and Werner demonstrated that the majority of patients studied had a rise in neutralizing antibodies against one of the strains isolated, the RI-67 strain (6).

The isolation of an agent, presumably a virus, from cases of ARD (6) immediately suggested an opportunity to establish the etiology of ARD and to test directly the hypotheses proposed by the Commission on Acute Respiratory Diseases (1–4). In addition, it was of interest to determine the relationship of the agents isolated by Rowe and co-workers (5), called "adenoid degeneration" or "A.D." agents, to the RI-67 agent. The results of experiments carried out to study these points are the subject of this report. A

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preliminary summary of certain of these data has been presented elsewhere (7).

MATERIALS AND METHODS

Viruses.[2] The viruses employed were the RI-67 strain of the "new acute respiratory disease virus" isolated by Hilleman and Werner and the prototype strains of Types I, II, and III "adenoid degeneration" agents isolated by Rowe, Huebner, Gilmore, Parrott, and Ward (5).

Pools of seed virus were prepared by passage in strain HeLa cell cultures (8) grown in 32 ounce Duraglas prescription bottles. For the RI-67 agent, 4 ml. of a 1:2 dilution prepared in Hanks balanced salt solution was inoculated onto a culture of HeLa cells. After 5 to 10 minutes at room temperature, 36 ml. of maintenance solution with 10 per cent chicken serum (8) was added. The adenoid degeneration agents, hereafter referred to as A.D. agents, were diluted 1:3.16 ($10^{-0.5}$) and 4 ml. inoculated onto each HeLa culture; the addition of 36 ml. of maintenance solution was subsequently made. Infected cultures were incubated at 36° C. for three to seven days until complete cellular degeneration had occurred. Clumped cells still adherent to the glass were removed with a rubber policeman on a curved glass rod, and these cells disintegrated in the infected supernatant fluid by grinding with a Teflon homogenizer (9) for two minutes. The homogenization chamber was kept chilled by an ice-water bath, and cell disruption was accomplished with the aid of a small quantity of alundum. The homogenized suspension was centrifuged at 2,500 rpm. for 10 minutes in a refrigerated International centrifuge, and the supernatant stored in sealed glass ampules at −70° C.

Sera. Lyophilized and liquid sera from donors and recipients of filtrates were available from the human transmission experiments carried out by the Commission on Acute Respiratory Diseases at Gatlinburg, Tennessee, in 1943 (10), and Pinehurst, North Carolina, in 1944 and 1945 (3, 4, 11). Lyophilized material had been stored at room temperature and liquid sera at 4° C. Sera obtained from hospitalized patients with clinical respiratory illnesses were also used for neutralization titrations. These sera had been stored in the lyophilized, frozen (−20° C.) or liquid (4° C.) state for varying periods since 1942. Many sera contained precipitated materials which were removed by centrifugation in the Spinco Model L centrifuge at 14,830 g for 15 minutes. All sera were heated at 56° C. for 30 minutes before use.

Red Cross pools of gamma globulin for antibody titrations were supplied by Dr. Glen Smith of the Cleveland Department of Health and by Dr. W. McD. Hammon of the University of Pittsburgh Graduate School of Public Health.

[2] The viruses were made available through the kindness of Dr. M. R. Hilleman, Army Medical Service Graduate School, and Dr. R. J. Huebner, Laboratory of Infectious Diseases, National Institutes of Health.

Tissue culture. The methods employed were modifications of those described by Scherer, Syverton, and Gey (8) for the propagation of the single cell line derived from an epidermoid carcinoma of the cervix of the uterus, designated strain HeLa. A HeLa cell culture was obtained from Dr. J. T. Syverton and has been maintained in serial passage for one year.

Cellular cultivation. Propagation of cells was carried out in bottles (200 ml. square screw-cap Kimble neutraglass, or 32 oz. flat-side Duraglas prescription, sealed with white rubber stoppers), or in 16 by 150 mm. screw-cap test tubes. Cells in bottles and tubes were incubated at 36° C. in a stationary position. Tubes were maintained at a 5° angle in specially constructed racks, and bottles were kept flat on the incubator shelves. For cultivation of cells a nutritive medium was employed composed of 25 per cent human serum, 2 per cent chick embryo extract (50 per cent), 5 per cent chick embryo ultrafiltrate (50 per cent),[3] and 68 per cent Hanks balanced salt solution. Penicillin and streptomycin were added to make a final concentration of 50 units and 0.5 mg. per milliliter of each, respectively. For preparation and distribution of cell suspensions, silicon coated glassware was used. With a rubber policeman placed on the curved end of a glass rod, HeLa cells were scraped from the bottle wall into 2 to 6 ml. of the clarified nutritive fluid in which they had been grown. Trypsin was not employed. The cellular sheets and clumps were disrupted by drawing back and forth through a 22 gauge needle attached to a 3 ml. Cornwall spring-activated syringe for 1.5 to 3 minutes. By this method a suspension was prepared composed almost entirely of single cells with only an occasional small clump of cells present. The number of cells per milliliter of suspension was determined by direct microscopic enumeration in a hemocytometer. The concentration of cells was adjusted to 80,000 per ml. by dilution with nutrient fluid and dispensed into bottles in 10 ml. (Kimble bottle) or 30 ml. (Duraglas bottle) volume with a pipette, or into test tubes in 0.7 ml. volume with a spring-activated Cornwall syringe. To obtain a complete sheet of cells in bottles required two to four days' incubation. Each day 5 ml. (Kimble bottle) or 15 ml. (Duraglas bottle) of nutrient fluid was removed and a like volume of fresh nutritive fluid was added. If essential, pH was adjusted to 7.6 to 7.8 with 1.4 per cent $NaHCO_3$. In tubes, HeLa cells formed into sheets and were ready to be infected within 24 hours in all but rare instances.

Infection of HeLa cell cultures. All nutritive fluid was removed as completely as possible and cultures were washed 2 times with Hanks balanced salt solution containing antibiotics. For bottle cultures each wash was done with 20 ml. or 50 ml. of fluid, and for tubes, 2 ml. per wash was employed. The liquid phase employed for infection was a synthetic fluid, maintenance solution (MS), containing 10 per cent chicken serum (8) and antibiotics. For test tube cultures, 0.8 ml. of MS plus

[3] Obtained from Microbiological Associates, Inc., Bethesda, Maryland.

chicken serum was employed; for bottles (Duraglas), 36 ml. was used.

Neutralization titration. Serial four-fold dilutions of each serum were prepared in Hanks balanced salt solution. To 0.3 ml. of each dilution was added an equal volume of virus diluted to contain 10 to 50 cytopathogenic tissue culture doses (TCD) per 0.1 ml. Serum-virus mixtures were incubated at room temperature (22 to 24° C.) for 30 minutes, after which period each of two tissue culture tubes was inoculated with 0.2 ml. of each serum dilution-virus mixture. The majority of these titrations was carried out with HeLa cell cultures in tubes purchased from Microbiological Associates, Inc. These tubes were prepared by methods similar to those described above. The results of neutralization and infectivity titrations in tubes prepared in either laboratory were similar, although a longer (two to four days) period of incubation was required to show maximum cytopathogenic changes in cultures made in this laboratory. Tubes were observed microscopically at least every two days and the final reading was made on the sixth day after inoculation. A culture was considered to be infected when round cells had developed throughout the culture and numerous cluster-like clumps of these cells had formed. In practically all titrations the end-point was sharp and the appearances of both tubes inoculated with a single serum dilution-virus mixture were similar. The end-point is expressed as the final dilution of serum in the serum-virus mixture which protected the cultured HeLa cells from viral cytopathogenic effects in both culture tubes. In the few instances in which protection was observed in only one of the two tubes, the extrapolation necessary to determine the exact end point was calculated. The proper serum and uninoculated tube controls were included in each experiment. All sera from a single individual were titrated on the same day.

Infectivity titration. Serial 0.5 $\log_{10}$ (1 : 3.16) dilutions were prepared in Hanks balanced salt solution, and to each culture tube was added 0.1 ml. of virus dilution. Two tubes per dilution were employed. The final observation of cultures was made on the sixth day after viral inoculation and the end-point is expressed as the initial dilution of virus which initiated specific cytopathogenic alterations of the HeLa cells in at least one of the two cultures. In the great majority of titrations changes were similar in both tissue culture tubes inoculated with a single dilution. When necessary, the proper calculations to determine the 50 per cent end-point (12) were made.

RESULTS

Neutralization titrations with sera from donors of respiratory secretions

Patients with illnesses considered to be clinically characteristic of primary atypical pneumonia (PAP), acute respiratory disease of recruits (ARD), bronchitis resembling atypical pneumonia (Br-AP), and cases of common cold, severe (S-CC) and mild (CC), were selected by the Commission on Acute Respiratory Diseases to provide infected respiratory secretions for human transmission experiments (3, 4). Sera from these patients had been saved and were available for this study. To determine whether the RI-67 agent isolated by Hilleman and Werner (6) was associated with any of these acute respiratory infections, neutralization titrations were carried out with this virus and the reconstituted lyophilized sera obtained during the acute and convalescent stages of the donors' illnesses. The results of these titrations are summarized in Table I. Development of antibodies against the RI-67 agent occurred in the patients who had ARD and bronchitis resembling atypical pneumonia, but not in those with primary atypical pneumonia or with the severe or mild forms of common cold. The

TABLE I

Results of neutralization titrations with sera from donors of respiratory secretions for human transmission experiments

Volunteer experiment	Clinical syndrome of donor	Number of donors	Number of rises in antibody titer
Gatlinburg 1943	Primary atypical pneumonia (PAP)	6	0
Pinehurst 1944	Primary atypical pneumonia (PAP)	6	0
Pinehurst 1945	Primary atypical pneumonia (PAP)	1	0
Pinehurst 1945	Acute respiratory disease (ARD)	1	1
Pinehurst 1945	Bronchitis resembling atypical pneumonia (Br-AP)	1	1
Pinehurst 1945	Common cold, mild (CC)	1	0
Pinehurst 1945	Common cold, severe (S-CC)	1	0

TABLE II

Neutralization titers of sera from donors in Pinehurst 1945 human transmission experiment

Clinical syndrome of donor*	Neutralization titer		Antibody rise
	Acute serum	Convalescent serum	
PAP	1:8	1:8	0
ARD	<1:8	1:32	+
Br-AP	<1:8	1:512 or >	+
CC	<1:8	<1:8	0
S-CC	1:8	1:8	0

* See Table I for designations.

exact titers of antibodies in sera from donors whose secretions were employed in the Pinehurst, 1945 experiment are presented in Table II. The significance of the high titer of antibodies which developed in the patient with bronchitis (Br-AP) as compared to the ARD donor is not clear. These data do suggest, however, that the etiologic agent or agents responsible for these two diseases were related to the RI-67 agent isolated by Hilleman and Werner (6).

Neutralization titrations with sera from recipients of ARD, primary atypical pneumonia, and severe common cold secretions

Transmission experiments carried out by the Commission on Acute Respiratory Diseases in 1945 indicated that the respiratory diseases designated as ARD, primary atypical pneumonia, and common cold were transmissible by bacteria-free filtrates of respiratory secretions and were indeed different entities (3, 4). Neutralization titrations carried out with sera from donors of these secretions against the RI-67 agent not only tended to confirm these findings, but also suggested that this new agent was etiologically associated with ARD. To extend these preliminary findings, titrations were carried out with sera from recipients in the 1945 transmission experiments. The results of these titrations are presented in Figure 1 along with the clinical results of the inoculation of control autogenous filtrates and infected materials from patients with ARD, primary atypical pneumonia and common cold (1, 4). A rise in neutralizing antibodies occurred only in individuals

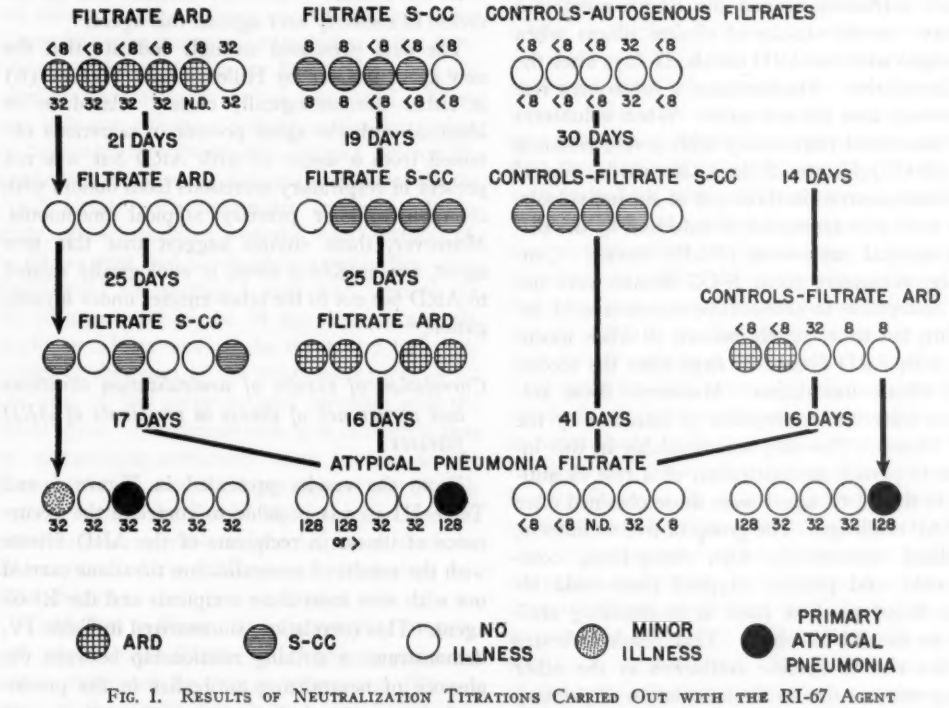

FIG. 1. RESULTS OF NEUTRALIZATION TITRATIONS CARRIED OUT WITH THE RI-67 AGENT AND SERA OBTAINED FROM HUMAN VOLUNTEERS BEFORE AND AFTER INOCULATION WITH FILTERED SECRETIONS FROM PATIENTS WITH ARD, SEVERE COMMON COLD (S-CC), OR PRIMARY ATYPICAL PNEUMONIA

Some recipients were also given filtered homologous secretions. Neutralization titers are indicated in relation to the appropriate inoculation. Each circle represents a volunteer and the shadings indicate the occurrence of illness. The sequence of events for each volunteer is represented by a single vertical column of circles.

who received ARD secretions and not in volunteers inoculated with autogenous, common cold or primary atypical pneumonia filtrates. In those instances in which sera were available two to three weeks following inoculation of ARD secretions, it was possible to demonstrate that antibodies developed in relation to the inoculation and, in most instances, concurrent with a clinical infection. Only two recipients failed to develop either detectable increase in antibodies or an illness; both had circulating antibodies at the time of intranasal inoculation of ARD secretions. There were three instances in which there was an increase in neutralizing antibodies directed against the RI-67 agent in the absence of a recognizable clinical illness; two of the individuals had antibodies when ARD secretions were inoculated. Those recipients initially inoculated with ARD filtrate who developed antibodies against the RI-67 agent, did not have second attacks of clinical illness when challenged with the ARD filtrate 21 days after the first inoculation. Furthermore, a subsequent rise in antibody titer did not occur. When volunteers were inoculated intranasally with severe common cold (S-CC) filtrate 25 days afterwards, clinical infections occurred in three out of six individuals. They were also susceptible to infection by the primary atypical pneumonia (PAP) filtrate. Conversely, volunteers given S-CC filtrate were not only susceptible to consecutive common cold infections, but subsequently became ill when inoculated with ARD filtrate 25 days after the second S-CC filtrate inoculation. Moreover, these volunteers were also susceptible to infection by the PAP filtrate. The only sera available in this instance to permit demonstration of a rise in antibody to the RI-67 agent were those obtained after the PAP challenge. The group of five volunteers, inoculated successively with autogenous, common cold, and primary atypical pneumonia filtrates, failed to show rises in neutralizing antibody to the RI-67 virus. This result indicates that the rise in specific antibodies in the other groups was not due to the inoculation of primary atypical pneumonia filtrate. Thus the data presented in Figure 1 show that the increase in immune bodies to RI-67 virus resulted from the ARD infection.

The results of neutralization titrations carried out on sera from all recipients inoculated with respiratory secretions from donors with ARD, severe common cold, or primary atypical pneumonia are summarized in Table III. These results add further evidence that individuals infected with secretions from a patient with ARD developed antibodies against the RI-67 agent, but those inoculated with secretions from patients with other acute respiratory diseases had no increase in antibody titer against this agent.

TABLE III

Results of neutralization titrations with sera from recipients in Pinehurst 1945 human volunteer transmission experiment

Disease of donor	Number of recipients	Number of rises in antibody titer
ARD	24	20
Atypical pneumonia	15	0
Common cold, severe	25	0

The data presented strongly indicate that the new agent isolated by Hilleman and Werner (6) is either immunologically closely related to or identical with the agent present in secretions obtained from a donor ill with ARD but was not present in respiratory secretions from donors with common cold or primary atypical pneumonia. Moreover, these studies suggest that this new agent, presumably a virus, is etiologically related to ARD but not to the other entities under investigation.

Correlation of results of neutralization titrations and occurrence of illness in recipients of ARD filtrates

From the results presented in Figure 1 and Table III it was possible to correlate the occurrence of illness in recipients of the ARD filtrate with the results of neutralization titrations carried out with sera from these recipients and the RI-67 agent. This correlation, summarized in Table IV, demonstrates a striking relationship between the absence of neutralizing antibodies in the pre-inoculation sera and the subsequent occurrence of illness; and conversely, between the presence of antibodies in sera obtained from recipients before intranasal inoculation and failure to manifest illness after inoculation of infected filtrate. Of the 24 volunteers inoculated, 18 or 75 per cent became

TABLE IV

Correlation of results of neutralization titrations with RI-67 agents and occurrence of illness in recipients of ARD secretions

Antibody response	Ill			Not ill		
	Antibody in pre-inoculation serum	No antibody in pre-inoculation serum	Total	Antibody in pre-inoculation serum	No antibody in pre-inoculation serum	Total
Rise	2	15	17	2	1	3
No rise	1	0	1	3	0	3
Total	3	15	18	5	1	6

ill; 15 or 83 per cent of these had no antibodies, and all developed neutralizing antibodies following inoculation. Only three (17 per cent) of those who became ill had antibodies in their pre-inoculation sera and two of the three had an increase in antibody titer in their post-inoculation sera. In sharp contrast were the six, or 25 per cent, of the volunteers who showed no evidence of clinical illness following intranasal inoculation of the ARD filtrate. Five of the six, or 83 per cent, had circulating immune bodies when inoculated. There was only one volunteer without antibodies at time of intranasal inoculation who did not become ill; antibodies did develop following exposure to the ARD filtrate. To summarize somewhat differently, of 24 inoculated with the ARD filtrate, 16, or 67 per cent, had no circulating antibodies when challenged; all developed antibodies against the RI–67 agent and 15, or 94 per cent, became ill. Eight, or 33 per cent, of the volunteers had antibodies at the onset of the experiment; five or 62 per cent, of this group resisted infection with the ARD agent. For comparison, it is of interest that no neutralizing antibodies were present in the pre-inoculation sera of 76 per cent of the 62 volunteers used in the Pinehurst, 1945 experiment, a percentage comparable to the 67 per cent of the 24 recipients of the ARD filtrate who had no immune bodies in their pre-inoculation sera.

These data indicate that as a result of inoculation of an ARD filtrate into human volunteers, illness developed in a large proportion (94 per cent) of those who did not have circulating neutralizing antibodies against the RI-67 agent. Furthermore, it may be concluded from these data that the presence in blood of antibodies against this virus is related to an undetermined degree to protection against the causative agent of ARD.

Neutralization titrations with sera from recipients of bronchitis resembling atypical pneumonia and mild common cold filtrates

Neutralization titrations carried out with the acute and convalescent phase sera from a patient with a disease syndrome termed "bronchitis resembling atypical pneumonia (Br-AP)" (3) exhibited a marked increase in antibodies against the RI-67 agent (Table I). Filtered respiratory secretions from this patient were inoculated into human volunteers and pre- and post-inoculation sera from the recipients were subsequently tested. Sera from individuals in the same experiment who were inoculated with secretions from a patient in the acute stage of a mild common cold (CC) were also titrated. The results of these neutralization titrations, as well as the occurrence of illnesses in the inoculated volunteers, are summarized in Figure 2. Only 4 of the 10 volunteers inoculated with Br-AP secretions developed a clinical illness. Sera were available from only three of these individuals, all of whom developed a small increase in neutralizing antibodies. The increase in titer demonstrable, although small, was considered to be significant as the titers were reproducible on repeated neutralization tests. Of the six recipients who showed no indication of infection following intranasal inoculation, four developed an increase in neutralizing antibodies; the rise in two instances was of greater magnitude than noted in sera from recipients with clinical illnesses. None of the recipients who received the common cold filtrate had an increase in neutralizing antibodies against the RI-67 agent. In addition, it will be noted that the recipients given the bronchitis filtrate were not subsequently resistant to the common cold, nor did homologous immunity develop in those who were given common cold filtrate

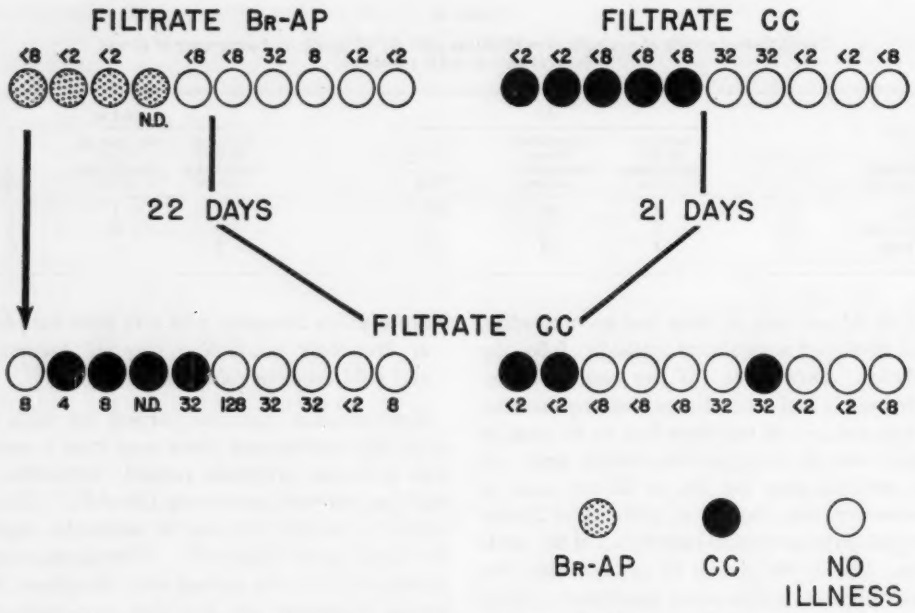
FILTRATE BR-AP
<8 <2 <2 N.D. <8 <8 32 8 <2 <2
FILTRATE CC
<2 <2 <8 <8 <8 32 32 <2 <2 <8
22 DAYS
21 DAYS
FILTRATE CC
8 4 8 N.D. 32 128 32 32 <2 8
<2 <2 <8 <8 <8 32 32 <2 <2 <8
BR-AP
CC
NO ILLNESS

FIG. 2. RESULTS OF NEUTRALIZATION TITRATIONS CARRIED OUT WITH THE RI-67 AGENT AND SERA OBTAINED FROM HUMAN VOLUNTEERS BEFORE AND AFTER INOCULATION WITH FILTERED SECRETIONS FROM ILLNESSES TERMED COMMON COLD (CC) OR "BRONCHITIS RESEMBLING ATYPICAL PNEUMONIA" (BR-AP)

The sequence of events in a single volunteer is represented by a single vertical column of circles. Each circle represents a volunteer and the shading indicates the occurrence of illness. Neutralization titers are indicated in relation to the appropriate inoculation.

initially and challenged with the same filtrate 21 days later.

A correlation of the results of the neutralization titrations and clinical illnesses in the recipients of "bronchitis resembling primary atypical pneumonia" (Br-AP) filtrate is presented in Table V. Although illness occurred only in volunteers who had no detectable RI-67 antibodies, a similar number of volunteers without circulating specific immune bodies in their pre-inoculation sera had no clinical infection following inoculation of the Br-AP filtrate. Two individuals who had RI-67 antibodies before inoculation of the Br-AP filtrate also remained well. From these data one can conclude that an agent immunologically related to the RI-67 agent was contained in the Br-AP filtrate, but these data do not indicate whether or not this agent was responsible for the

TABLE V

Correlation of results of neutralization titrations with RI-67 agent and occurrence of illness in recipients of bronchitis resembling atypical pneumonia (Br-AP) secretions

Antibody response	Ill			Not ill		
	Antibody in pre-inoculation serum	No antibody in pre-inoculation serum	Total	Antibody in pre-inoculation serum	No antibody in pre-inoculation serum	Total
Rise	0	3	3	1	3	4
No rise	0	0	0	1	1	2
Total	0	3	3*	2	4	6

* No sera were available from one volunteer who became ill (see Figure 2).

clinical infections observed in the four recipients who became sick. The relatively few illnesses which were induced in these volunteers, as well as the extreme mildness of the infection (3), may have influenced these results considerably.

Results of neutralization titrations with sera from ARD epidemics at military installations

Thus far, ARD has been recognized only as a disease which occurs in epidemics in military recruits. To obtain further evidence concerning the etiologic relationship of the epidemic respiratory disease, ARD, to the RI-67 agent isolated by Hilleman and Werner (6), sera from soldiers who experienced ARD in epidemics which occurred at three different military installations (Fort Bragg, North Carolina; Warren Air Force Base, Wyoming; and Sampson Air Force Base, New York) from 1942 to 1953 were titrated for neutralizing antibodies. The results of these titrations, summarized in Table VI, indicate that numerous cases of ARD at Fort Bragg and Sampson Air Force Base in 1942 and 1953, respectively, were associated with and probably caused by the RI-67 agent. It is clear, however, that all illnesses termed ARD were not related to this virus. At Fort Bragg, 1944 and 1945, and Warren Air Force Base, 1949 and 1951, although a rise in titer of neutralizing antibodies directed against this agent was noted in occasional cases, the majority of those examined had no increase in these specific immune bodies.

Several different interpretations may be attached to these data: 1) The clinical syndrome termed ARD may be caused by a number of unrelated viruses; 2) a single class of agents may induce ARD but more than one immunological type may exist; 3) during a sharp single wave epidemic a single immunologic type may be implicated, whereas at other times a number of types may compete in the production of sporadic cases; or 4) the RI-67 agent occasionally may propagate and stimulate the production of specific antibodies in patients with ARD, although it is not the specific causative agent of the clinical disease observed. The evidence available suggests that the last interpretation is unlikely, but does not permit exclusion of any of the other possibilities.

TABLE VI

Results of neutralization titrations with sera from ARD patients at military installations

Camp	Year	Antibody rise
Ft. Bragg	1942	10/12*
	1944	8/35
	1945	1/12
Warren AFB	1949	1/3
	1951	0/4
Sampson AFB	1953	13/21

* Numerator = number antibody rise; denominator = number tested.

Neutralization titrations with pooled gamma globulin and RI-67 agent

An estimation of the prevalence of the RI-67 agent in the civilian population could be readily obtained by the determination of the presence of antibodies directed against it in gamma globulin prepared from pooled Red Cross plasma. Seven pools were tested; all contained neutralizing antibodies in titers of 1:32 (1 pool) or 1:128 (6 pools). Although in low dilutions (1:8 or less) each sample employed had a deleterious effect upon HeLa cells, this did not interfere with the end-point determination which in every instance was at least four-fold higher than the toxic effect of the gamma globulin. One may deduce from these data that the RI-67 agent or one immunologically related to it does propagate in human hosts in the civilian population, and that either infections by this agent occur frequently, or antibodies which develop as a result of infection persist for a relatively long period of time.

Neutralization titrations with A.D. agents (Rowe and co-workers) and sera from donors and recipients in 1945 human transmission experiment

Evidence already presented suggests that the RI-67 agent isolated by Hilleman and Werner (6) is etiologically related to the disease entity ARD. From tissues obtained at routine tonsillectomies and adenoidectomies, Rowe and co-workers isolated a number of agents which, in HeLa cell tissue cultures, appeared to have certain characteristics similar to the RI-67 agent (5). It was therefore of interest to compare these agents in neutralization titrations to determine: 1) whether antibodies against the A.D. agents

TABLE VII

Results of neutralization titrations with Type II A.D. agent and sera from donors of secretions for Pinehurst, 1945 experiment

Syndrome*	Titer		Rise
	Acute serum	Convalescent serum	
PAP	<1:8	<1:8	0
ARD	1:8	1:8	0
Br-AP	1:8	1:32	+
CC	<1:8	<1:8	0
S-CC	<1:8	<1:8	0

* See Table I for designations.

developed during the clinical infections under study; and 2) the immunological relationship between the RI-67 and A.D. agents. The results of titrations carried out with the Type II agent and sera from donors of respiratory secretions in the 1945 experiment are presented in Table VII. A small increase in circulating antibodies occurred in the donor with "bronchitis resembling primary atypical pneumonia" but not in donors who had ARD, primary atypical pneumonia, or common cold, mild or severe. Titrations were next carried out with sera from all recipients who received the Br-AP filtrate as well as with sera from a number of recipients who received the ARD filtrate and had an increase in antibody titer against the RI-67 agent. From the results of these titrations with the A.D. Type II agent, summarized in Table VIII, it is noted that a rise in antibody titer occurred in only 1 of 13 recipients who received ARD filtrate and 1 out of 9 volunteers inoculated with the Br-AP filtrate. In each instance the titer increase was from 1:8 in the acute phase serum to 1:32 in the convalescent serum. It should be emphasized that all pairs of sera tested from ARD filtrate recipients and 7 of the 9 pairs from volunteers given Br-AP had a rise in antibody to the RI-67 agent. When Types I and III A.D. agents became available there were insufficient quantities of these sera to perform further titrations. Paired sera from eight other recipients of ARD filtrate, however, were tested to determine whether an increase in antibodies against the Type I and III agents occurred concomitantly. All of these recipients had a clinical illness and manifested a rise in the RI-67 neutralizing antibody titer. In not a single instance could a rise in antibody titer against either A.D. agent be demonstrated. In addition sera obtained from cases of ARD at Sampson Air Force Base in 1953 were titrated for antibodies against the 3 types of agents. Although 13 out of 21 showed an increase in titer for the RI-67 virus (Table VI), there were no rises for the A.D. agents. Neutralization titrations carried out with other sera, from human cases and immunized animals (13), clearly indicated that there was no immunological relationship demonstrable by this technique between the RI-67 agent isolated by Hilleman and Werner (6) and the 3 immunological types of A.D. agents obtained from tonsils and adenoids by Rowe, Huebner, Gilmore, Parrott, and Ward (5). Moreover, there was no increase in neutralizing antibodies to the A.D. agents in sera from two patients with respiratory infections observed during the winter of 1954 in which a rise in neutralizing antibodies directed against the RI-67 agent occurred. Conversely, in a single instance in which an increase in antibodies directed against Type III agent occurred, immune bodies specific for the RI-67 virus did not develop. By complement-fixation titrations, however, an antigenic relationship between the RI-67 and A.D. agents can be demonstrated (6, 13—See Addendum).

TABLE VIII

Results of neutralization titrations carried out with Type II A.D. agent and sera from recipients of ARD and bronchitis (Br-AP) filtrates

Recipients given filtrate	Rise in Type II neutralizing antibody titer
ARD	1/13*
Br-AP	1/9

* Numerator = number showing antibody rise; denominator = number tested.

DISCUSSION

The serological studies presented demonstrate that, in sera from a donor of filtered ARD respiratory secretions as well as recipients of this filtrate, there developed neutralizing antibodies directed against the RI-67 agent isolated by Hilleman and Werner (6); that illness occurred in the great majority of recipients who had no antibodies in their pre-inoculation sera; that a rise in antibody titer occurred in all recipients in whom the pre-inoculation sera were devoid of neutralizing antibodies; and that the majority of recipients

whose pre-inoculation sera contained neutralizing immune bodies were resistant to infection and did not have a further increase in neutralizing antibodies. The donor with the infection termed "bronchitis resembling atypical pneumonia," which clinically could not be easily distinguished from ARD, also had a rise in titer of antibodies to the RI-67 agent. Moreover, seven of the nine recipients of filtered respiratory secretions from this donor developed antibodies against this agent, but one could not directly correlate the occurrence of illness and rise in antibody titer with the presence or absence of antibodies in the pre-inoculation sera. Insufficient data are available to permit clear interpretations of these serologic results. Neutralizing antibodies to the RI-67 agent did not develop in either donors or recipients of common cold or primary atypical pneumonia filtrates.

It might be expected that the presence of an agent in secretions inoculated into humans would stimulate antibody formation against this agent if propagation of the agent ensued. This series of events does not alone provide conclusive evidence that illnesses which occurred in recipients subsequent to inoculation of these secretions were causally effected by the agent in question. The excellent correlation of absence of antibodies in pre-inoculation sera with occurrence of illness, and antibody rise, and conversely, the presence of circulating immune bodies at time of inoculation with resistance to infection and lack of antibody development, constitute strong evidence that the agent in question actually initiated the infection. Such was the case with ARD and the RI-67 agent. The fact that this agent or one closely related to it immunologically was associated with epidemics of ARD and not with epidemics of other acute respiratory infections is further evidence that the RI-67 agent isolated by Hilleman and Werner from cases resembling ARD (6) is indeed the etiologic agent, or at least one of the agents, which causes this respiratory infection. The data available, although still meagre, do suggest that other agents, possibly biologically closely related but immunologically different, also initiate epidemic and sporadic cases of this disease.

The question inevitably arises relative to what criteria are necessary to prove the etiologic relationship of a new agent to a clinical disease. For this purpose, many deem it essential to reproduce the infection in humans by inoculation of the new agent. It is proposed however, that it should be possible to obtain sufficient data from detailed clinical and epidemiological studies correlated with laboratory investigation to establish the etiologic relationship of a newly isolated agent with most infectious diseases. The data of importance might be categorized as follows: 1) Isolation of the agent should be possible from the majority of patients with the disease in question; the agent should be obtained from infected tissue or from secretions related to this tissue; 2) Specific antibodies directed against the new agent should develop in relation to infection; and 3) Resistance to infection should be correlated with presence of type specific, neutralizing antibodies; conversely, susceptibility should be related to absence or low titer of these specific antibodies. Perhaps the most definitive evidence in relation to these three points can be obtained when the disease in question occurs in a sequential or epidemic fashion in defined population groups. Data on each of these points have been obtained with the RI-67 agent and ARD and presented by Hilleman and Werner (6) and the present investigation.

Although the epidemic of acute respiratory disease studied by Hilleman and Werner (6) was that of an influenza-like illness considered to be ARD, the RI-67 strain of cytopathogenic agents was isolated from a patient who had a pulmonary infiltration. This infection was diagnosed to be primary atypical pneumonia although a rise in cold hemagglutinins or agglutinins to streptococcus MG did not occur (14). The data presented (Table I, Figure 1) indicate that in hospitalized patients and human volunteers a number of classical cases of primary atypical pneumonia of the type associated with a rise of cold hemagglutinins was not caused by the RI-67 agent. In addition, a number of hospitalized cases considered clinically to be primary atypical pneumonia in whom no cold hemagglutinins or streptococcus MG agglutinins developed were investigated; none had a rise in titer of neutralizing antibodies against the RI-67 agent. Of 22 cases of ARD which occurred at Sampson Air Force Base in 1953, pulmonary infiltration by x-ray was noted in four cases. Three of these patients manifested an increase in RI-67 neutralizing antibodies. Further laboratory and detailed clinical studies concerning these ill-

nesses were not available. These data suggest that pulmonary infiltration may develop during ARD. In the absence of information relative to the etiology of these infections, these cases would be termed primary atypical pneumonia. With knowledge of the causal agent, a specific diagnosis can be made. That pulmonary infiltration unassociated with bacterial infection may develop during the course of an upper respiratory viral disease has been amply demonstrated in influenza infections (15, 16).

The role of the group of viruses isolated by Rowe, Huebner, and co-workers (5) as causative agents of disease is not yet clear. It is certain, however, that these viruses are different from the RI-67 agent and do not cause the infection, ARD, studied in the present investigation. That upper respiratory infections of similar nature may be caused by one of these agents is possible. Moreover, the data suggest that these viruses are not the etiologic agents of primary atypical pneumonia or common cold infections. Evidence is available, however, that the A.D. agents are to some extent immunologically related to the RI-67 agent: although antigenic resemblances cannot be detected by neutralization techniques, complement-fixation titrations reveal that the 3 types of A.D. agents and RI-67 virus are immunologically related by a common antigen (6, 13—See Addendum).

The results of the serological studies presented confirm the conclusions of the Commission on Acute Respiratory Diseases that ARD is a distinct clinical upper respiratory disease and that the infection confers an homologous immunity. These data further indicate that although ARD studied in the human transmission experiments was initiated by a single etiologic agent, similar infections which occurred in epidemics were caused not only by this agent, RI-67, but by others still unidentified. It is therefore proposed that agents which are shown to produce the clinical infection recognized as ARD be called ARD viruses; the RI-67 virus, as well as others which may subsequently be isolated, falls into this classification.

SUMMARY

Sera obtained from donors of infected respiratory secretions and recipients of these secretions during a human transmission experiment carried out by the Commission on Acute Respiratory Diseases, 1945, were employed for neutralization titrations with the RI-67 agent isolated by Hilleman and Werner (6) and the A.D. agents obtained by Rowe and his co-workers (5). These studies indicated that the acute upper respiratory infection, ARD, was caused by an agent, presumably a virus, identical with or closely related to the RI-67 agent; that the A.D. agents did not produce this illness; and that ARD is a clinical entity distinct from the common cold or primary atypical pneumonia. In addition to the RI-67 agent, ARD is probably caused by other agents, immunologically different from the RI-67 virus. It is proposed that the RI-67 agent be called an ARD virus.

Addendum

Subsequent to the submission of this paper for publication an article entitled "Adenoidal-Pharyngeal-Conjunctival Agents. A newly Recognized Group of Common Viruses of the Respiratory System," by Huebner, R. J., Rowe, W. P., Ward, T. G., Parrott, R. H., and Bell, J. A. appeared (New England J. Med., 1954, **251**, 1077) in which the Adenoid Degeneration Agents and the RI-67 virus isolated by Hilleman and Werner were classified as a single group. This was termed the Adenoidal-Pharyngeal-Conjunctival group of viruses. In this report and the paper which accompanied it (Parrott, R. H., Rowe, W. P., Huebner, R. J., Bernton, H. W., and McCullough, N. M., New England J. Med., 1954, **251**, 1087) some of the characteristics of these agents were described, the relationship of the complement-fixing and neutralizing antibodies was indicated, and the isolation of the Type III agent from cases of acute febrile pharyngitis was reported.

ACKNOWLEDGMENT

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REFERENCES

1. Commission on Acute Respiratory Diseases, Acute respiratory disease among new recruits. Am. J. Pub. Health, 1946, **36**, 439.
2. The Commission on Acute Respiratory Diseases, Clinical patterns of undifferentiated and other acute respiratory diseases in army recruits. Medicine, 1947, **26**, 441.
3. The Commission on Acute Respiratory Diseases, Experimental transmission of minor respiratory illness to human volunteers by filter-passing agents. I. Demonstration of two types of illness charac-

terized by long and short incubation periods and different clinical features. J. Clin. Invest., 1947, **26**, 957.

4. The Commission on Acute Respiratory Diseases, Experimental transmission of minor respiratory illness to human volunteers by filter-passing agents. II. Immunity on reinoculation with agents from the two types of minor respiratory illness and from primary atypical pneumonia. J. Clin. Invest., 1947, **26**, 974.
5. Rowe, W. P., Huebner, R. J., Gilmore, L. K., Parrott, R. H., and Ward, T. G., Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exper. Biol. & Med., 1953, **84**, 570. Introduced by E. Verder.
6. Hilleman, M. R., and Werner, J. H., Recovery of new agent from patients with acute respiratory illness. Proc. Soc. Exper. Biol. & Med., 1954, **85**, 183.
7. Dingle, J. H., Ginsberg, H. S., Badger, G. F., Jordan, W. S., Jr., and Katz, S., Evidence for the specific etiology of "acute respiratory disease (ARD)." Tr. A. Am. Physicians, 1954, **67**, 149.
8. Scherer, W. F., Syverton, J. T., and Gey, G. O., Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix. J. Exper. Med., 1953, **97**, 695.
9. Pierce, C. H., Dubos, R. J., and Schaefer, W. B., Multiplication and survival of tubercle bacilli in the organs of mice. J. Exper. Med., 1953, **97**, 189.
10. The Commission on Acute Respiratory Diseases, An experimental attempt to transmit primary atypical pneumonia in human volunteers. J. Clin. Invest., 1945, **24**, 175.
11. Commission on Acute Respiratory Diseases, The transmission of primary atypical pneumonia to human volunteers. I. Experimental methods. Bull. Johns Hopkins Hosp., 1946, **79**, 97.
12. Reed, L. J., and Muench, H., A simple method of estimating fifty per cent endpoints. Am. J. Hyg., 1938, **27**, 493.
13. Ginsberg, H. S., Unpublished data.
14. Horsfall, F. L., Jr., Primary atypical pneumonia. Ann. Int. Med., 1947, **27**, 275.
15. Scadding, J. G., Lung changes in influenza. Quart. J. Med., 1937, n.s., **6**, 425.
16. Scher, J. M., and Jaruszewski, E., Virus influenza A′ infection with pulmonary manifestations. Arch. Int. Med., 1952, **90**, 201.

THE ISOLATION AND ELECTROPHORETIC CHARACTERIZATION OF THE PROTEINS IN THE URINE OF NORMAL SUBJECTS[1]

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Studies of urinary proteins in disease characterized by proteinuria have been carried out by numerous investigators using a variety of techniques including electrophoresis (1–3). Although it is now generally conceded that protein is found in the urine of normal individuals (4), it is present in such small quantities as to escape detection by ordinary procedures. Except for the work of Rigas and Heller (5) a survey of the literature does not reveal any definitive data on the nature of the proteins found in the urine of normal individuals. These authors, on isolation and concentration of the high molecular weight material present in normal urine by ultrafiltration, reported that the material was electrophoretically separable into components the mobilities of which corresponded to those of albumin and of the various globulins usually found in serum.

Rigas and Heller (5) found the A/G ratio in the urine of normal individuals to be 0.51 reflecting relative preponderance of globulins. They commented on the heterogeneity of globulin components and suggested it could have been acquired during the ultrafiltration process. The work reported here deals with an attempt to establish whether the low A/G ratio and the heterogeneity of the globulins were artifacts of the method used by Rigas and Heller. Their method and other methods for concentrating proteins from dilute solutions were compared. For evaluation of the methods a dilute solution of normal serum in 0.9 per cent saline was used. The effect, if any, of a method of concentration on the electrophoretic pattern could thus be judged, using the pattern of the untreated serum as a standard.

The introduction of zone electrophoresis had made it possible to separate small amounts of protein mixtures into their electrophoretic components (6, 7). The method has been applied by Slater and Kunkel (3) to the characterization of proteins in pathological urine by paper electrophoresis. However, they reported inability to isolate and characterize proteins from normal urines. An attempt to identify the albumin found in normal urine with that of the serum is also reported here.

MATERIALS AND METHODS

All operations, unless otherwise stated, were carried out in a cold room, the temperature of which was 2° ± 1° C. For evaluation of methods a dilute solution of normal serum was used (1 ml. of serum per liter of saline) and this will be referred to as dilute serum in saline. Two liters of this solution were used for each determination.

When experiments with normal urines were undertaken a set of concurrent experiments was carried out on each of the following solutions:

(I) a dilute solution of serum proteins in saline as above,
(II) an aliquot of normal urine,
(III) a similar aliquot of the same normal urine to which was added 1 ml. of normal serum per liter.

In each set of experiments the same normal serum was used. Control sera were fresh pooled samples obtained through cooperation of the Blood Bank of the Royal Victoria Hospital. In all experiments the electrophoretic pattern of the untreated serum was independently determined by free electrophoresis.

Pooled urine specimens were collected at midday from young adult men and women working in the laboratories of the University Clinic. The subjects for the experiments on individual urines were all young adult men.

All urines were placed in the refrigerator immediately after voiding and were filtered as soon as possible. They were found free of protein by the conventional heat and acetic acid test. The final step in each method was the same: dilution, solution or suspension in veronal buffer to a volume of about 12 ml. and dialysis against veronal for at least 24 hours before electrophoretic analysis was carried out. After dialysis, using the refractive

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[2] Senior Fellow of the Canadian National Research Council.

index of the buffer solution, "protein" concentration was calculated from the refractive index increment as measured with an Abbe refractometer (8). For consistency the same method was used for estimating the concentration of protein recovered from serum in saline solutions and from urine. In the latter instance it is considered only an approximation of the total protein content since one does not know the specific refractive index for urinary proteins or the contribution to the refractive index increments of non-protein high molecular weight material present in urine. In four instances the solution was recovered from the cell and, after dialysis against saline until the dialysate was found free of barbiturate, the protein nitrogen was measured chemically with a modified micro-Kjeldahl method and the protein calculated using the factor of 6.25. Barbiturate was measured by the method of Koppanyi, Murphy, and Krop (9) which is sensitive to 0.05 mg. per cent. Protein nitrogen was determined in duplicate on the precipitate formed by phosphotungstic acid.

Electrophoretic analysis: Because of the greater accuracy of free electrophoresis (classical Tiselius method) as compared to paper electrophoresis (10), the former technique was used for evaluation of methods of protein recovery. Free electrophoresis was carried out in an improved Tiselius apparatus [3] in which a clearly defined pattern of serum proteins can be obtained with as little as 0.5 per cent protein solution. All experiments were carried out in veronal buffer of pH 8.6 and ionic strength 0.1. In general, green monochromatic light (wavelength = 5,461Å) was used; in some cases, when urines were deeply colored, a yellow filter had to be used. Conductivities were measured with an LKB bridge [4] at 0° C. Mobilities were calculated from the descending limb using the method of Longsworth and MacInnes (11). Using the method of calculation of Tiselius and Kabat (12) concentrations were obtained by planimetry of the areas of only the ascending pattern for consistency since in the descending pattern of normal sera per cent distribution could not be calculated because of the beta anomaly. It may be mentioned that in this laboratory only one fasting serum has failed to show the presence of the anomaly in over 500 analyses. Paper electrophoresis was done by the "horizontal strip" technique as modified in this laboratory (13).

Pervaporation: Concentration of dilute serum in saline solution by pervaporation was attempted. Five hundred ml. aliquots were placed in dialysis tubing (Visking —3 in. wide) and hung at room temperature in a current of air produced by electric fans. After reduction in volume by about 100 ml., the bags were dialyzed against running tap water in order to prevent the concentration of salts as well as that of protein. These operations were done alternatively until the volume was reduced to about 50 ml. Final concentration to about 10 ml. was achieved by dialysis against dextran. Lyophylization was not attempted because it would have resulted in concentration of salts as well as proteins.

Dialysis against dextran: It was thought feasible to concentrate dilute protein solutions, concurrently removing water and salts, by dialysis against a concentrated aqueous solution of a high molecular weight material. Technical grade dextrans, which became available through the courtesy of various commercial firms were used for this purpose. A 25 per cent solution could readily be made with the dextran supplied by Commercial Solvents Corporation [5] (average molecular weight 60,000) whereas the Swedish dextran [6] with similar specifications was not as soluble. Three hundred to 350 ml. aliquots of serum in saline solution were placed in dialysis bags and dialyzed against dextran solution until the volume in each bag was about 30 ml. The contents of six bags were then pooled in one bag and dialysis against dextran continued until the volume was reduced to about 10 ml. Concentration of urine and urine plus serum was attempted on one occasion using Swedish dextran. During the long period of dialysis a good deal of small molecular weight dextran entered the bags so that the final volume could not be reduced below 30 ml.

Ammonium sulfate precipitation: Two attempts were made to apply the classical method of salting out with ammonium sulfate by saturating dilute serum in saline solution with the salt. Following precipitation the salts were removed by dialysis against running tap water and the precipitate was so finely divided that even centrifugation at 15,000 rpm. for 20 minutes did not result in a clear separation. Starting with 2 liters of serum in saline solution the procedure outlined above required at least two weeks. It was considered impractical and abandoned.

Ultrafiltration—Negative pressure: An apparatus such as is described by Dent (14) was used with the exception that bags were made from Visking dialysis tubing instead of collodion.

Ultrafiltration—Positive pressure: This method was used by Rigas and Heller in their experiments (5). For the present study an apparatus, such as is described by Gorbman (15), was built. A commercial model was also available and was used. It was deemed preferable to use membranes of constant pore size. Using unglazed cellophane, Visking cellulose membrane and Visking dialysis membrane and a pressure of 80 to 100 lbs. per sq. in., passage of distilled water was limited to a few ml. per hour. With Gottingen "Ultrazellafilter" membranes distilled water passed through at a rate of about 100 ml. per hour. Distilled water passed through collodion membranes prepared in this laboratory at a rate of about 10 ml. per min. It was felt that elution of the protein from the membrane would be preferable to dissolution of the membrane. However, attempts at elution, using 0.9 per cent NaCl or veronal buffer of pH 8.6 and ionic strength

[3] Spinco Model H, Specialized Instruments Corporation, Belmont, California.

[4] Supplied by LKB Produckter, Stockholm, Sweden.

[5] Supplied through the courtesy of Commercial Solvents Corporation, Terre Haute, Indiana.

[6] Supplied through the courtesy of Pharmacia, Uppsala, Sweden.

of 0.1 were unsuccessful. An attempt to displace the protein by reversing the membrane and passing 0.9 per cent NaCl or veronal buffer through it under pressure also failed. Therefore it was necessary to dissolve the membranes even though the steps necessary were considered to be harsh treatment in view of the ease with which proteins are denatured. Collodion membranes were used and, after completion of filtration, were dissolved in alcohol-ether, washed three times with alcohol-ether, once with ether and dried. In the case of serum in saline, the precipitate was then dissolved in veronal buffer and dialyzed against veronal buffer before the electrophoretic analysis. The method of Rigas and Heller (5) prescribed the following additional steps when urine was used. The precipitate was extracted three times with 1.0 M saline. The pooled supernatant fluid was dialyzed against water for twenty-four hours following which saturated saline solution was added (2 ml. of saline for every 10 ml. of supernatant) and the proteins precipitated with 5 vol. of 94 per cent alcohol. Following centrifugation the precipitate was washed with absolute alcohol, once with anhydrous ether and dried before dissolving in veronal buffer.

Alcohol precipitation: Since repeated use of alcohol-ether solution was found necessary to recover proteins from the membranes after ultrafiltration, a step resulting in precipitation of the proteins as well as dissolution of the membrane, it was decided to precipitate the proteins directly with alcohol. One liter aliquots of a solution of serum in saline were placed in dialyzing jars which were packed in ice to bring down the temperature to 0° C. Five volumes of 94 per cent ethyl alcohol were added slowly from a separatory funnel with constant stirring of the solution. Addition of the first liter at a rate greater than about 60 drops per min. resulted in a sharp rise in temperature to 8 to 10° C. due to the heat of mixing. Addition of the alcohol at 50 to 60 drops a min. limited the temperature rise to 1 to 2° C. After addition of the first liter the alcohol was added at the rate of about 600 ml. per hr. Since no suitable refrigerated centrifuge to cope with such large volumes was available at the beginning of these experiments, the solution was left standing in the cold room until the precipitate was down and the supernatant clear. This usually occurred in 48 to 72 hours. The supernatant was then removed and the remainder transferred to a cylinder from which additional supernatant could be removed after 2 to 4 days. The precipitate was then centrifuged at 15,000 rpm. for 15 mins. in a Spinco Model L refrigerated ultracentrifuge. The precipitate was suspended in veronal.

Precipitates obtained by applying this method to urine, or to urine plus serum were too bulky to be dissolved in 12 ml. of veronal. At first they were suspended in distilled water (100 to 150 ml. being required) and dialyzed against distilled water. They were then dialyzed against saline in order to assure solution of any euglobulins which might have precipitated out during dialysis against distilled water. The contents of the dialysis bag were centrifuged and the supernatant removed. To this were added 5 volumes of alcohol dropwise. The solution was allowed to stand until the precipitate came down, usually 48 to 72 hours. The supernatant was suctioned off and the remainder spun down at 15,000 rpm. The precipitate was then suspended in veronal buffer.

Because of the bulk of the original precipitate it was felt that more efficient extraction of the protein from it might be obtained if the precipitate were suspended repeatedly in saline. Extractions were repeated until a negative test for protein was obtained with the heat and acetic acid test. This test is sensitive to 2 mg. per cent (16). Since 15 to 20 ml. of saline were used for each extraction subsequent to the second one, the amount of protein lost could not exceed, at the most, 0.4 mg. or less than 0.3 per cent of the total in any one experiment. The supernatants from each extraction, after centrifugation, were pooled and then dialyzed against saline for at least 48 hours with repeated changes of saline. In order to lessen the period of contact of protein with alcohol and to minimize the degree of denaturation while still maintaining the temperature of resultant mixture within reasonable limits, precooled alcohol was used. It was found that when alcohol, precooled to a temperature of − 40° C., was added at about 100 ml. per min., the temperature of the mixture during addition of the first liter rose to 4° C. The final temperature of the mixture after addition of 5 volumes of alcohol ranged from − 10 to − 14° C.

Ideally it would be desirable to centrifuge immediately after addition of alcohol, in the initial and final precipitation. Near the end of this study a more suitable refrigerated centrifuge (International PR2) became available and centrifugation immediately after the final addition of alcohol was carried out in the last 14 experiments and serum recoveries from saline were somewhat better. It was also done with the initial precipitations in the two final experiments. Final precipitates were completely soluble in veronal. It might be mentioned that the addition of precooled alcohol to a solution of serum in saline did not result in immediate precipitation of the proteins so that centrifugation had to be postponed for several days. However, precipitation became evident immediately after addition of precooled alcohol to urine and the supernatants after centrifugation remained completely clear indefinitely. Immediate centrifugation of the suspensions cut down the time required for the procedure from 3 to 4 weeks to 6 days.

For further support of the validity of this method for concentrating urinary proteins and for the identification of the component having the fastest electrophoretic mobility (which appeared to be that of albumin) the following experiments were done. An aliquot of I^{131} tagged human serum albumin,[7] representing 24 microcuries and 0.3 mg. of albumin, was added to each of the following: 2 liters of serum in saline solution, urine, and to urine plus serum solution and these three solutions were run through the procedure concurrently. The urine used represented, in each case, a 60-hour aliquot of a 5-day collection in one individual. The serum came from the

[7] Obtained from Abbott Laboratories.

same pool of normal serum. Electrophoresis was done in the Tiselius apparatus and on paper. For paper electrophoresis a more concentrated protein solution is required than for free electrophoresis. Therefore, an amount of the veronal solution of the final precipitate, representing a 20-hour aliquot in the case of urine and an equivalent amount in the case of the serum in saline solution, was concentrated by dialysis against a 50 per cent solution of dextran [8] to a volume of about 0.25 ml. This concentrate was applied to paper, 0.01 ml. being used in the case of the urine concentrate, 0.006 ml. in the case of the serum in saline concentrate. Electrophoresis was done at room temperature using 150 volts for 13 hours. The papers were stained with Amidoschwartz 10B, cut in 0.5 cm. strips and each strip placed in a tube. Counting was done in a scintillation well counter consisting of a crystal of sodium iodide 1½ inches in diameter and 2 inches deep. Sensitivity of this counter is 8×10^5 counts per minute per microcurie and is essentially independent of volume so that activity in each strip was counted completely. After counting, the dye was eluted from each strip with a solution of 50 per cent methanol containing 5 ml. in NaOH per liter (17) and read in a Beckman Model B spectrophotometer at 600 mμ.

In an attempt to further identify the "albumin" found in urine with that of serum, I^{131} tagged human serum albumin (IHSA) was injected intravenously into two normal individuals. The dose used in each case contained 1 mg. of albumin and 26 microcuries. Blood specimens were taken at 12 minutes, 24 and 48 hours after injection. Urine was collected for 48 hours. The concentrated urine proteins were characterized electrophoretically in the Tiselius apparatus and on paper. In view of the fact that the dose of IHSA was, of necessity, limited in human subjects, larger amount of protein had to be applied to paper. To achieve this the protein solution was concentrated by dialysis against 50 per cent dextran to about three times the protein concentration found in normal serum and 0.3 ml. of this solution was applied linearly with a pipette to three superimposed sheets of Whatman 3 mm. filter paper 6¾ inches wide. The filter papers were supported on a sheet of plexiglass 7 inches wide and 14 inches long. The surface of the plexiglass was pebbled in a regular fashion with small trapezoid projections (6 to the square inch). The sheet of plexiglass with the papers were sandwiched between frames. These were enclosed between glass plates the inner surfaces of which were completely covered by sheets of Whatman 3 mm. filter paper dipping into the buffer vessels. The buffer used was veronal and the ionic strength 0.05, voltage was 160 V, the current per strip was 2.5 to 3 mA. The resulting bands were clearly and equally resolved on each of the three papers and did not show any deviation from linearity. The papers were cut into strips 0.5 cm. wide. The protein curves were determined as before. I^{131} counts were done on 2 strips (*i.e.*, 1 cm.) at a time because of the small amount of IHSA present.

[8] Provided through the courtesy of the R. K. Laros Company, Chemical Division, Bethlehem, Penna.

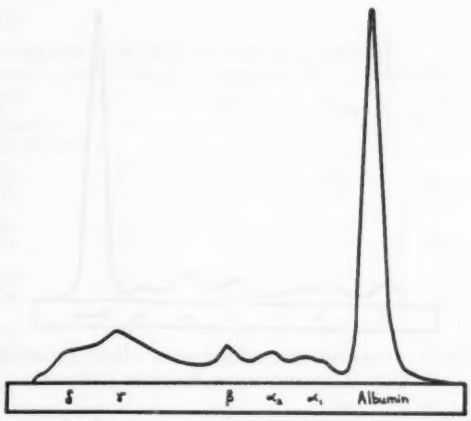

FIG. 1. CONCENTRATION OF SERUM IN SALINE SOLUTION BY PERVAPORATION

RESULTS

Serum in saline

Examples of typical electrophoretic patterns obtained by the various methods applied to the serum in saline solution may be seen in Figures 1 to 6 and compared with that of an untreated serum shown in Figure 7.

The Schlieren pattern obtained with pervaporation was not satisfactory. There was an additional peak in the region of alpha-1 and it was not possible, in either the ascending or descending limb, to clearly separate the gamma globulin peak from the sigma and epsilon anomalies (Figure 1).

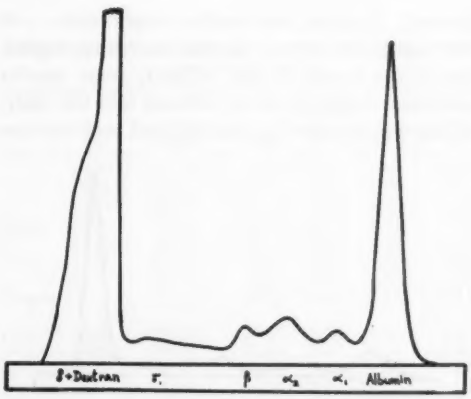

FIG. 2. CONCENTRATION OF SERUM IN SALINE SOLUTION BY DIALYSIS AGAINST DEXTRAN SOLUTION

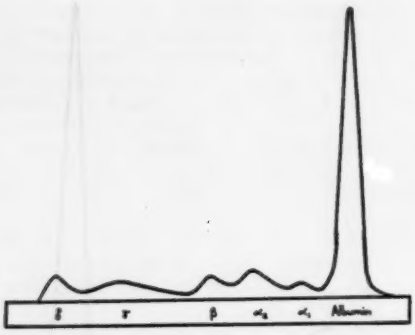

FIG. 3. CONCENTRATION OF SERUM IN SALINE SOLUTION BY ULTRAFILTRATION WITH NEGATIVE PRESSURE

Concentration against dextran gave fairly good results on one occasion when technical grade dextran, supplied by Commercial Solvents Corporation, was used (Figure 2). With the Swedish dextran no individual globulin peaks could be de-

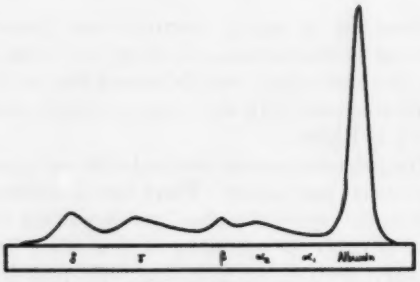

FIG. 4. CONCENTRATION OF SERUM IN SALINE SOLUTION BY ULTRAFILTRATION WITH POSITIVE PRESSURE

lineated. In these and in other experiments with technical grade dextrans in this laboratory, regardless of the source of the polymer, some smaller molecular weight material diffused into the dialysis bag and appeared as an additional peak between

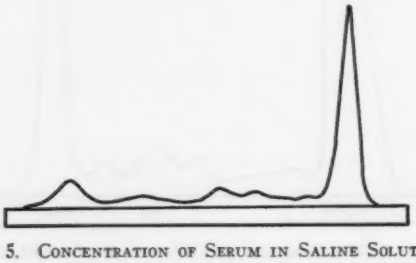

FIG. 5. CONCENTRATION OF SERUM IN SALINE SOLUTION BY A SINGLE PRECIPITATION WITH ALCOHOL

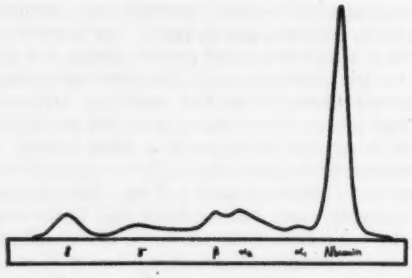

FIG. 6. CONCENTRATION OF SERUM IN SALINE SOLUTION BY PRECIPITATION WITH ALCOHOL FOLLOWED BY EXTRACTION WITH SALINE AND FURTHER PRECIPITATION WITH ALCOHOL

the gamma and the delta and epsilon anomalies. In the experiment with the dextran supplied by Pharmacia this peak was exceedingly high.

Ultrafiltration by negative pressure gave good results (Figure 3). It required, however, 18 to 21 days to concentrate 2 liters of serum in saline solution by this method. The time required to concentrate a similar volume of urine would obviously be longer because of the greater solute content found in urine and, therefore, this method was considered impractical.

Ultrafiltration by positive pressure using collodion membranes was done using 2 liter aliquots of serum in saline solution (Figure 4). All fractions could usually be delineated by this method but quantitative deviations occurred from one experiment to another. For example, in some experiments the percentage of albumin was greater than in the original pattern, on other occasions it was less. The best recovery obtained by this method was 53.7 per cent. The average recovery

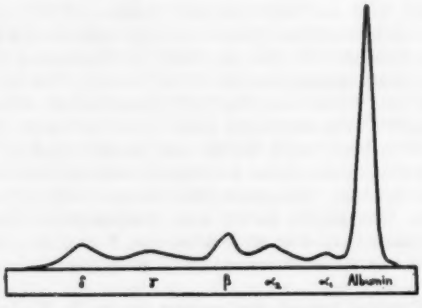

FIG. 7. TYPICAL CURVE FOR CONTROL SERUM

TABLE I

Electrophoretically determined composition of untreated sera and of sera recovered from saline solution *

Exp. No.	% Protein†	Albumin %	Alpha 1 %	Alpha 2 %	Beta %	Gamma %	A/G ratio‡	% Recovery
			By alcohol precipitation at 0° C.					
U.S. 226	0.90	56.01	5.23	10.96	13.47	14.32	1.27	
R.S. 260	0.87	63.74	5.33	9.50	9.31	12.12	1.76	60.4
U.S. 293	0.50	53.70	5.78	11.47	14.16	14.89	1.15	
R.S. 310	0.54	68.36	4.93	12.34	4.69	9.67	2.16	48.9
U.S. 316	0.94	57.02	4.85	11.26	13.97	12.90	1.33	
R.S. 408	0.92	63.45	4.21	9.08	10.61	12.64	1.74	72.4
U.S. 404	1.05	58.65	3.17	10.58	12.02	15.58	1.42	
R.S. 430	0.94	63.90	3.68	10.52	8.45	13.44	1.77	69.7
Average U.S.		56.50	4.76	11.07	13.41	14.42	1.30	
Average R.S.		64.86	4.54	10.36	8.27	11.97	1.86	62.9
			By alcohol precipitation at −40° C. and extraction					
U.S. 404	1.05	58.65	3.17	10.58	12.02	15.58	1.42	
R.S. 448	0.66	72.38	3.15	7.79	8.52	7.97	2.62	46.8
R.S. 487	0.74	57.21	5.35	20.88	8.37	8.18	1.34	57.8
U.S. 512	0.98	58.38	3.90	10.98	11.85	14.88	1.4	100.0
R.S. 490	1.03	62.26	3.24	14.59	9.98	9.92	1.65	70.0
R.S. 523		66.33	4.54	10.74	8.65	9.73	1.97	
Average U.S.		58.52	3.54	10.78	11.94	15.23	1.41	
Average R.S.		64.55	4.07	14.29	8.88	8.97	1.89	68.7

* The electrophoretic pattern of each serum recovered from dilute saline solution (R.S.) is compared with the electrophoretic pattern of the original untreated serum (U.S.).
† Protein concentration in Tiselius cell.
‡ The A/G ratio for individual normal fasting sera in this laboratory is 1.5 ± 0.17. The control sera in these experiments were pooled p.c. specimens.

on the last four experiments, when it was felt the technique was mastered, was 48.6 per cent.

Alcohol precipitation

This method gave the best recoveries of protein and the most consistent electrophoretic results. It was the only method by which all serum components in the dilute serum solution could be clearly and consistently separated. In general, there was an increased proportion of albumin in the pattern of the recovered serum when compared to that of the untreated serum, while there was a decrease in the beta and gamma globulins; the alpha globulins for the most part showed little change. Figure 5 illustrates the pattern obtained for recovered serum by a single alcohol precipitation of the protein followed by dissolution in veronal buffer. Figure 6, on the other hand, represents the pattern obtained when the initial precipitate was extracted with saline and reprecipitated with alcohol. These additional steps had been found necessary for the characterization of the urinary proteins. The relatively high albumin recovery by this method might be expected. In Cohn's method of plasma fractionation (18) al-

TABLE II

Electrophoretic composition of urine in normal individuals and urine + serum

	Exp. No.	Albumin	Globulins			A/G ratio
			Alpha	Beta	Gamma	
Urine	449	42.58	37.31	11.24	8.86	0.74
	464	32.13	46.91	9.19	11.76	0.37
	489*	53.99	31.69	7.51	6.80	1.20*
	524	39.74	42.28	11.47	6.51	0.66
	522	46.22	31.45	15.13	7.20	0.86
	558	40.30	37.73	14.20	7.77	0.68
	547	35.83	45.59	12.00	6.57	0.56
Average		39.13	40.21	12.21	8.11	0.65
Urine + serum	450	58.42	27.67	6.44	7.46	1.4
	488	59.35	22.84	10.06	7.74	1.46
	525	56.56	28.90	7.97	6.56	1.30
	519	51.29	30.28	8.19	10.24	1.05
Average		54.2	28.5	9.24	8.03	1.2

* Not included in average, see Text.

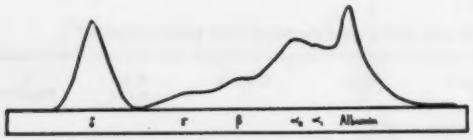

FIG. 8. CONCENTRATION OF NORMAL URINE BY ALCOHOL PRECIPITATION

bumin is the last component of the plasma proteins to be precipitated. Under the conditions of these experiments albumin appears to be the most soluble component. This was demonstrated in an experiment in which the pooled supernatants of the first three saline extractions were halved and to one were added the supernatants of the 4th and 5th extractions. Protein recovered by these additional extractions consisted mainly of alpha and beta globulin; it contained only 6.3 per cent albumin and 4.45 per cent gamma globulin. Table I summarizes the results of the alcohol precipitation method applied to the recovery of serum proteins from saline solutions.

Urines

The results obtained by applying the alcohol precipitation method to normal urine are presented in Table II. An example of the Schlieren patterns obtained with such concentrates of urine

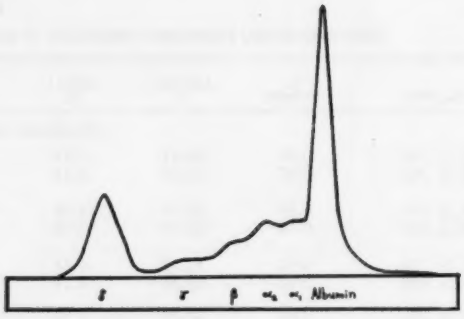

FIG. 9. CONCENTRATION OF SERUM IN NORMAL URINE BY ALCOHOL PRECIPITATION

and of the same urine to which normal serum had been added is shown in Figures 8 and 9.

Electrophoretic mobilities of the various peaks in the Schlieren patterns of urines were of the same order of magnitude as the mobilities obtained in this laboratory for the recognized serum proteins (Table III). The significance of these data will be dealt with in the discussion. It may be pointed out, however, that the various components have been arbitrarily labelled as the ones conventionally found in serum.

It is evident that albumin makes up a smaller percentage of the protein found in urine as compared with serum proteins. The preponderant

TABLE III

Mobilities of electrophoretically separable components found in the urine of normal individuals*

	Exp. No.	Albumin	Alpha		Gamma	
			Alpha 1	Alpha 2	Beta	Gamma
	410	6.58		5.62	3.63	2.22
	431	6.50		5.20	3.45	2.27
	449	6.75		5.29	3.59	2.10
	464	6.70		5.28	3.60	2.37
	465	6.69	5.59	5.03	3.52	2.54
	489	6.62		5.39	3.80	2.43
	522	6.58		4.96	3.44	2.72
	524	6.58	5.39	4.98	3.62	2.39
	Average	6.62		5.29	3.58	2.38
	S.D.	±0.08		±0.21	±0.11	±0.19
	Range	6.50–6.75		4.96–5.62	3.44–3.80	2.10–2.72
Normal† sera	Average‡	6.73	5.76	4.50	3.54	1.67
	S.D.	±0.20	±0.18	±0.14	±0.23	±0.18
	Range	6.43–7.18	5.48–6.16	3.82–5.06	3.22–4.03	1.42–2.01

* These values have been divided by 10^{-5} cm.^{2} per sec. per volt.

† These values are given for comparison; they are based on experiments of untreated fasting sera obtained from 14 normal subjects.

‡ The mobilities are slightly higher than those conventionally quoted (19). They were observed at a serum protein concentration of only 0.5 to 1 per cent. These results are in agreement with the findings of Lippman and Banovitz (20).

globulins are those having a mobility distribution similar to that of the alpha globulins of serum. This confirms the findings of Rigas and Heller (5).

The A/G ratio was found to be, with one exception, Exp. No. 489, Table II, less than one, with an average of 0.65 compared to an average of 1.5 in serum (by the electrophoretic method). Two individuals with urinary A/G ratios greater than unity were found in the study by Rigas and Heller (5) to have orthostatic albuminuria. In the present study evidence of the existence of orthostatic albuminuria was looked for but was not obtained; no albuminuria was present in this subject after activity. However, the results of the experiments with the urine of this subject were not included in the calculation of the averages.

The amount of protein found in the urine averaged 70.9 mg. per day when measured refractometrically. Four chemical determinations of protein concentration gave values ranging from 6.9 per cent to 12.3 per cent (average 10.1 per cent) lower than those obtained refractometrically.

The results of the experiments in which I^{131} tagged human serum albumin was added to serum in saline, to urine and to urine plus serum are shown in Figure 10. The I^{131} peak is confined to an area which corresponds with the albumin peak of the protein curve. It may be seen that the I^{131} peak is slightly ahead of the albumin peak in agreement with the recent report of Gabrieli, Goulian, Kinersly, and Collet (21). Figure 10 also demonstrates that the result obtained when urinary proteins are separated by electrophoresis on paper is similar to that obtained with free electrophoresis. The amount of protein applied to the paper in this instance represents 1.3 per cent of the total recovered from a 60-hour urine specimen, that is, the amount excreted in 50 minutes.

When I^{131} tagged human serum albumin was injected intravenously into two normal human subjects the dose was limited to 26 microcuries and the amount of tagged albumin excreted was correspondingly small. One cm. strips were counted for 10 minutes. Counts from regions other than the albumin area gave values below background. The increments in count of the

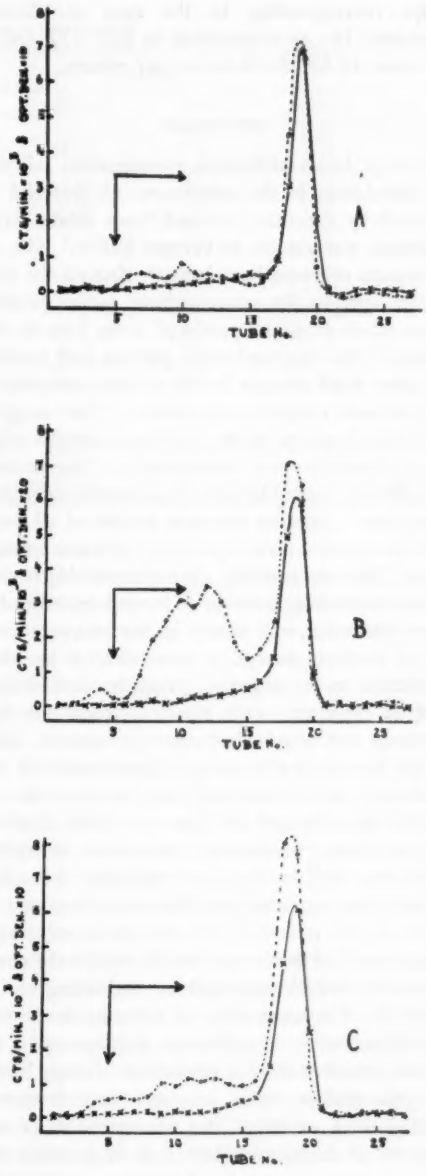

FIG. 10

Crosses represent I^{131} counts and circles represent optical densities. *Curves A* refer to concentration of serum in saline solution containing I^{131}-HSA. *Curves B* refer to concentration of urine containing I^{131}-HSA. *Curves C* refer to concentration of serum in urine containing I^{131}-HSA.

strips corresponding to the area of albumin amounted in one experiment to 525 ± 77 and in the other to 520 ± 78 counts per minute.

DISCUSSION

Except in ultrafiltration experiments, followed by dissolution of the membrane, all material recovered by the other method from dilute serum solutions was soluble in veronal buffer. The experiments reported here indicate that all the techniques applied for concentrating serum proteins from dilute solutions produced some loss of definition of the electrophoretic pattern and resulted in some small change in the relative amounts of the various protein components. This suggests that the alteration in the Schlieren pattern might be attributed to some denaturation of the proteins on dilution, a well-known phenomenon in protein chemistry. Another common feature of all methods is a lower recovery of the globulin component. The beta anomaly, almost invariably present in the descending patterns of human serum in free electrophoresis, was absent in the recovered sera by all methods except by concentration by ultrafiltration under negative pressure and dialysis against dextran; with these methods the beta anomaly was detectable but not prominent. Most of the lipoproteins in serum migrate with the beta globulin. It is accepted that lipoproteins are highly unstable and the low recoveries of globulin, in these experiments regardless of method used, may well be due to the splitting of the lipoprotein bond on dilution of the serum in saline. In spite of this it is felt that the dilute solution of serum used in these experiments fulfills the conditions of a reliable standard for evaluating various methods of concentration of proteins in urine.

Concentration by alternate pervaporation and dialysis resulted in poor resolution. It may be that dialysis against water was not done frequently enough and, at times, the concentration of salts rose to an undesirable level. It is probable that this may also account for the failure of Slater and Kunkel to characterize proteins in normal urine following concentration by lyophylization (3).

During concentration by dialysis against dextran, salts as well as water were removed. At the same time, however, some of the smaller molecular weight dextrans present in the batches used entered the dialysis bag. This prevented the concentration process from proceeding to the extent required for electrophoretic experiments. At the time of these experiments no dextrans of a more homogeneous molecular weight distribution (*ca.* 40,000) were available. It was impossible to concentrate the urines without preventing diffusion of the smaller molecular weight dextrans into the urine solutions. Further experiments were therefore abandoned. In principle, however, there is no reason why concentration of urine by dialysis against a concentrated aqueous solution of an adequate polyelectrolyte should not yield satisfactory results. As a matter of fact, towards the end of this study dextrans of a more homogeneous molecular weight distribution became available.[9] With a 30 to 50 per cent solution of these dextrans dilute protein solutions can be almost completely dehydrated. These dextrans were used for concentrating the solutions recovered from the Tiselius cell prior to application to paper in the I^{131} studies.

Ultrafiltration by positive pressure did not prove satisfactory in our hands. The pore size of collodion membranes almost certainly varies from one membrane to another. It is conceivable that on occasion smaller protein molecules may escape through the membrane. This may account for the comparatively low A/G ratio for recovered serum proteins of 1.21 obtained with this method.

It has been demonstrated that passage of urine through a membrane decreases its permeability to water (15). This blocking effect is greater with urines containing readily demonstrable protein. Apparently protein adheres to the interstices of the membrane. We were unable to elute the proteins from the membrane by washing with saline or veronal buffer or by reverse flow under pressure. To recover the proteins the membrane was dissolved in alcohol-ether solution, which is considered to be drastic treatment for proteins in general. Additional steps necessary to recover proteins from urine required further precipitation of the proteins with alcohol (5). It seemed logi-

[9] Provided through the courtesy of L. K. Laros Company, Chemical Division, Bethlehem, Penna. (Batch No. IV—370–361–372, mol. wt. 74,000; 10 per cent low fraction of mol. wt. 30,100) and the Dextran Corporation, Yonkers, N. Y. (Batch No. 2058, average mol. wt. 58,800).

TABLE IV

Electrophoretic mobilities of serum proteins recovered from saline*

	Exp. No.	Albumin	Globulins			
			Alpha 1	Alpha 2	Beta	Gamma
	408	6.84	5.80	4.43	3.72	1.67
	448	6.65	5.57	4.34	3.48	1.75
	463	6.81	5.83	4.93	3.66	1.68
	487	6.58	5.71	3.93	3.62	1.72
	490	6.49	5.60	3.99	3.62	1.86
	515	6.67	5.72	4.03	3.51	1.69
	523	6.58	5.60	4.35	3.59	1.86
	Average	6.66	5.69	4.29	3.60	1.74
	S.D.	±0.12	±0.10	±0.34	±0.08	±0.08
	Range	6.49–6.84	5.57–5.83	3.93–4.43	3.48–3.72	1.67–1.86
Normal† sera	Average‡	6.73	5.76	4.50	3.54	1.67
	S.D.	±0.20	±0.18	±0.14	±0.23	±0.18
	Range	6.43–7.18	5.48–6.16	3.82–5.06	3.22–4.03	1.43–2.01

* These values have been divided by 10^{-5} cm.^{2} per sec. per volt.

† These values are given for comparison; they are based on experiments of untreated fasting sera obtained from 14 normal subjects.

‡ The mobilities are slightly higher than those conventionally quoted (19). They were observed at a serum protein concentration of only 0.5 to 1 per cent. These results are in agreement with the findings of Lippman and Banovitz (20).

cal, therefore, to precipitate the proteins directly with alcohol.

The alcohol precipitation method gave the most consistent results and the best recoveries of serum proteins from dilute solutions. It is not ideal. There was always some loss of definition of the globulin peaks in the Schlieren pattern. The method favours the recovery of albumin. The A/G ratio of serum recovered from saline solution was always higher than that found in untreated sera. Nevertheless, it is to be noted that electrophoretic mobilities of the various components in the recovered sera closely approximated the normal values (Table IV) which is considered strong support in favour of this method of concentration. Experiments at different pH and ionic strength might give more satisfactory results.

Schlieren diagrams obtained when the alcohol precipitation method was applied to urine showed poor resolution. Peaks of the slower moving components are superimposed on a continuous broad curve. The area under any one peak in the globulin region is somewhat arbitrarily delineated and its measurement and the per cent composition derived from it are at best only an approximation. The Schlieren diagrams presented in the paper by Rigas and Heller (5) reveal a similar loss of definition of the globulins in normal urines. However, the albumin peak in both studies is relatively well-separated from the globulins and the A/G ratio can be obtained with a fair degree of accuracy. When the ultrafiltration method was applied to serum in saline in this laboratory the A/G ratio of the recovered proteins was found to be 1.21 compared to 1.75 found with the alcohol precipitation method. The electrophoretically determined A/G ratio of the untreated sera used in these experiments was 1.36. It is not surprising, therefore, that the A/G ratio of 0.65 found in this study is slightly higher than that of 0.51 reported by Rigas and Heller.[10] Nonetheless, the results of these authors showing a reversed A/G ratio in normal urines as compared to that found in normal sera are confirmed by the present experiments.

In spite of poor delineation of the Schlieren pattern electrophoretic mobilities of the components found in urine are remarkably reproducible (Table III). The mobilities of albumin and of globulins labelled alpha and beta in urine are of the same order of magnitude as of their counterparts in serum. However, the peak between beta globulin and the delta and epsilon anomalies, respec-

[10] Since the submission of the present paper for publication, Boyce, Garvey, and Norfleet (22) have reported that using the method of Rigas and Heller they obtained a value of 0.65 for the A/G ratio in normal urines, which is identical to our value. They also stated that the average amount of colloids excreted per 24 hours was 86 to 97 mg. which is in excellent agreement with the values of 90 mg. derived in the present report.

tively, labelled as gamma globulin, has a higher mobility than that of gamma globulin in serum. The predominant globulins in normal urine are those having a mobility similar to the alpha globulins of serum.

In both ascending and descending patterns there was a marked increase in the size of the delta and epsilon anomalies, respectively. Rigas and Heller found (5) this to be due to a high molecular weight polysaccharide. These authors also reported (5) an unidentified component migrating more rapidly than albumin which they designated X. Similarly, in all the urine experiments in the present study there was some material with a greater mobility than that of albumin. This was seen as a small peak in the initial stages of the electrophoretic resolution but became ill defined in the final pattern probably due to diffusion. No attempt was made to further characterize this or the material contributing to the enlargement of the delta and epsilon peaks. It might be mentioned that in the electrophoretic analysis of cerebrospinal fluid proteins a small peak occurs ahead of the albumin (23).

Such a peak is also found in this laboratory when serum proteins are separated by zone electrophoresis using starch as a supporting medium. Similarly, it has been reported to be present in certain abnormal sera in free electrophoresis (24).

Calculation of total protein concentration of urine from the refractive index increment can only give an approximation as has already been pointed out. Total protein content based on nitrogen determination was found to be 10 per cent lower than that found by refractive index measurements. The data reported here by either method are too meager to draw many conclusions.

For convenience, in this study actual figures have been used to express recoveries, composition, and mobilities. These absolute values must be considered with caution.

Thus, the techniques employed have qualitative and quantitative limitations and the results of the experiments on urines can only be interpreted accordingly. Within this frame of reference they are believed to be valid.

A survey of the literature does not reveal any recent direct determination of the daily output of proteins in the urine of normal individuals. The results of Mörner (25) led to a value of 25 to 89 mg. per liter. Rigas and Heller state the average daily output to be 39 mg. (5) as calculated from their electrophoretic patterns. Unfortunately, it is not possible to derive from their published data what their per cent recovery was. The results of the present study, using the alcohol method show the average daily output to be 71 mg. This may be considered the lower limit since the experiments with dilute saline solution gave only an average recovery of 70 per cent. Assuming that the per cent recovery from urines was of the same order, it would seem probable that the actual value for the average daily output could be as high as 90 mg. based on nitrogen determination.

While the absolute amounts of the protein components recovered when serum is added to urine may be questioned, examination of the Schlieren diagrams of these experiments shows that neither the heterogeneity nor the preponderance of globulins in normal urine can be attributed to the limitations of the method.

The I^{131} studies support the thesis that the albumin found in urine is identical with that of plasma, in fact it is, in part at least, serum albumin.

Assuming all the proteins found in urine are serum proteins filtered through the glomerular membrane, the relatively low albumin concentration can be readily explained by tubular reabsorption (4). There is no absolute evidence, however, that all of the albumin and the globulins found in normal urine are derived solely from serum proteins. Immunological studies and further isotope studies with labelled serum proteins other than albumin would help to clarify the situation.

It is conceivable that some of the protein found in normal urine could be contributed by breakdown of cells shed from the genito-urinary tract.

It is equally possible that the Schlieren pattern which appears to be characteristic of urine in normal individuals may be the result of interaction between the proteins present and other high molecular weight material known to be present in urine. Rigas and Heller suggest as a possible explanation for the heterogeneity of the patterns the "co-existence of proteins with intermediate-electrophoretic mobilities which are either absent from serum or present in very small amounts" (5). This would imply relative failure of reabsorption or tubular secretion of these moieties.

The few experiments using paper electrophoresis have shown that this simpler and cheaper method of analysis could be fruitfully applied to evaluation of the protein pattern in urines. The advantage of this method over free electrophoresis is that it requires much smaller aliquots of urine. On one occasion a 12-hour aliquot proved to be adequate. It also enables one to localize isotope tagged protein fractions and could be used to differentiate the glyco and lipoprotein moieties, in urine.

SUMMARY

Methods for concentrating proteins from dilute solutions are described and their limitations are discussed. Alcohol precipitation was found to give the best recoveries and the most consistent results. The proteins in the urine of normal subjects, isolated by the alcohol precipitation method, were characterized by free electrophoresis. Protein components were found in urine having mobilities of the same order of magnitude as albumin and globulins of serum. The preponderant globulins of urine were those having an electrophoretic mobility similar to that of the alpha globulins of serum. Globulin components showed marked heterogeneity. The A/G ratio was found to be 0.65. An unidentified moiety with a mobility greater than that of albumin was found to be present in urine. The average protein output in the urine of normal individuals would appear to be at least 71 mg. a day, a figure somewhat higher than previously reported. Experiments with I^{131} tagged human serum albumin in two normal subjects showed that serum albumin is excreted in the urine of normal individuals. Some experiments using paper electrophoresis demonstrated that this method may be applied to the study of electrophoretic pattern of proteins in relatively small aliquots of normal urine.

ACKNOWLEDGMENTS

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REFERENCES

1. Luetscher, J. A., Jr., Electrophoretic analysis of plasma and urinary proteins. J. Clin. Invest., 1940, **19**, 313.
2. Longsworth, L. G., and MacInnes, D. A., An electrophoretic study of nephrotic sera and urine. J. Exper. Med., 1940, **71**, 77.
3. Slater, R. J., and Kunkel, H. G., Filter paper electrophoresis with special reference to urinary proteins. J. Lab. & Clin. Med., 1953, **41**, 619.
4. Rather, L. J., Filtration, resorption and excretion of protein by the kidney. Medicine, 1952, **31**, 357.
5. Rigas, D. A., and Heller, C. G., The amount and nature of urinary proteins in normal human subjects. J. Clin. Invest., 1951, **30**, 853.
6. Durrum, E. L., A microelectrophoretic technique and microionophoretic technique. J. Am. Chem. Soc., 1950, **72**, 2943.
7. Kunkel, H. G., and Tiselius, A., Electrophoresis of proteins on filter paper. J. Gen. Physiol., 1951, **35**, 89.
8. Armstrong, S. H., Jr., Budka, M. J. E., Morrison, K. C., and Hasson, M., Preparation and properties of serum and plasma proteins. XII. The refractive properties of the proteins of human plasma and certain purified fractions. J. Am. Chem. Soc., 1947, **69**, 1747.
9. Koppanyi, T., Murphy, W. S., and Krop, S., Colorimetric detection of barbital and its applications. Proc. Soc. Exper. Biol. & Med., 1933, **30**, 542.
10. Köiw, E., Wallenius, G., and Grönwall, A., Paper electrophoresis in clinical chemistry; a comparison with Tiselius' original method. Scandinav. J. Clin. & Lab. Invest., 1952, **4**, 47.
11. Longsworth, L. G., and MacInnes, D. A., The interpretation of simple electrophoretic patterns. J. Am. Chem. Soc., 1940, **62**, 705.
12. Tiselius, A., and Kabat, E. A., An electrophoretic study of immune sera and purified antibody preparations. J. Exper. Med., 1939, **69**, 119.
13. Grassmann, W., and Hannig, K., Ein quantitatives Verfahren zur Analyse der Serumproteine durch Papierelektrophorese. Hoppe Seyler's Ztschr. f. physiol. Chem., 1952, **290**, 1.
14. Dent, C. E., Paper chromatography and medicine *in* Recent Advances in Clinical Pathology. S. C. Dyke, ed., 2nd ed., London, J. & A. Churchill, 1951, p. 238.
15. Gorbman, A., Ultrafiltration of urine for collection and biological assay of excreted hypophyseal hormones. Endocrinology, 1945, **37**, 177.
16. Ham, T. H., ed., A Syllabus of Laboratory Examinations in Clinical Diagnosis; Cortical Evaluation of Laboratory Procedures in the Study of the Patient. Cambridge, Mass., Harvard Univ. Press, 1950, p. 496.
17. Pučar, Z., Über die Färbung der Papier-Elektropherogamme mit Amidoschwartz. Hoppe Seyler's Ztschr. f. physiol. Chem., 1954, **296**, 62.

18. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues aad fluids. J. Am. Chem. Soc., 1946, **68**, 459.

19. Dole, V. P., The electrophoretic patterns of normal plasma. J. Clin. Invest., 1944, **23**, 708.

20. Lippman, R. W., and Banovitz, J., The influence of protein concentration upon electrophoretic mobility of serum proteins. J. Biol. Chem., 1952, **199**, 451.

21. Gabrieli, E. R., Goulian, D., Jr., Kinersly, T., and Collet, R., Zone paper electrophoresis studies on radio-iodinated human serum albumin. J. Clin. Invest., 1954, **33**, 136.

22. Boyce, W. H., Garvey, F. K., and Norfleet, C. M., Jr., Proteins and other biocolloids of urine in health and in calculous disease. I. Electrophoretic studies at pH 4.5 and 8.6 of those components soluble in molar sodium chloride. J. Clin. Invest., 1954, **33**, 1287.

23. Hoch, H., and Chanutin, A., Electrophoretic studies on cerebrospinal fluid. Proc. Soc. Exper. Biol. & Med., 1952, **81**, 628.

24. Hoch, H., and Chanutin, A., Electrophoretic studies on fast moving components of human serum. J. Biol. Chem., 1953, **200**, 241.

25. Mörner, K. A. H., Untersuchungen über die Proteinstoffe und die eiweissfällenden Substanzen des normalen Menschenharns. Skandinav. Arch. f. Physiol., 1895, **6**, 332.

CHANGES IN FREE AMINO ACIDS IN THE PLASMA DURING HEPATIC COMA

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Previous reports from various laboratories have emphasized the failure to demonstrate consistent and significant biochemical changes during hepatic coma (1–3). However, a gross increase of amino acids in the plasma, urine and spinal fluid (4, 5) and an elevation of ammonia in the blood (6–9) have been observed to accompany hepatic coma. Since diseases of the liver often result in disturbances of protein metabolism, which are usually manifested by a change in the concentration of amino acids in the plasma, it was felt that a quantitative study of individual amino acids in patients in various stages of hepatic coma might provide some information to elucidate the relationship between the biochemical and clinical findings. The results of such a study, which are presented in this report, indicate that the content of free amino acids in the blood was usually increased during severe hepatic disease. Increases in some or all of

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TABLE I

Summary of the case histories of the patients

Case	Age (yrs.)	Sex	Coma			Condition of patient
			Pre-coma	Light	Deep	
1	65	M	X			Acute viral hepatitis; moderate jaundice; condition fairly good and patient never beyond drowsy state; complete recovery.
2	65	M	X			Same as case 1.
3	62	F	X			Acute viral hepatitis; deep jaundice; urea measured 12 mg. per 100 cc. of blood.
4	33	F	X			Stricture of common bile duct; appeared normal; some vomiting and hematemesis (?); stupor day after blood samples were taken.
5	54	M		X		Carcinoma of common bile duct; deep jaundice with urea of 200 mg. per 100 cc. of blood; electrolytes normal; died day after blood samples were taken.
6	33	F			X	Stricture of common bile duct; deep coma after hemorrhage; died with convulsions next day.
7	45	M			X	Portal cirrhosis; electrolytes normal; mild jaundice; died 5 days after withdrawal of blood.
8	33	F			X	Stricture of common duct; normal values for urea and electrolytes; electroencephalogram showed typical changes of deep hepatic coma; patient was conscious next day.
9	41	F			X	Postnecrotic cirrhosis; deep coma; had been given 300 mg. of cortisone day before blood was withdrawn; values for urea and electrolytes normal; died next day.
10	62	F			X	Acute viral hepatitis; very deep jaundice; died next day.
11	32	M			X	Postnecrotic cirrhosis; had repeated blood transfusions; electrolytes normal; deep jaundice; urea measured 78 mg. per 100 cc. of blood; in deep coma 20 days before blood withdrawn; died next day.
12	34	M			X	Postnecrotic cirrhosis; deep coma after portocaval shunt; never regained consciousness after operation done 3 days before blood was taken; died soon after blood was taken.

TABLE II

Free amino acids and related compounds of the plasma

Compound	Normal†	Hepatic coma*												
		Precoma						Deep coma						
		Cases				Amino acid increased, cases, number	Light coma Case 5	Cases						Amino acid increased, cases, number
		1	2	3	4			6	7	8	9	10	11	12
Alanine	36.7 (24.3–50.3)	0.7	0.9	1.6	0.8	1	0.2	0.8	0.7	0.7	1.1	3.7	2.1	8.5	3
Cystine	6.1 (3.5–7.5)	1.9	3.0	1.3		2	8.6	0.6	1.8	1.0	3.0	1.8	4.9	5.4	5
Glutamic acid	10.1 (8.9–11.4)	1.5	0.9	1.5	1.7	3	2.2	0.6		1.4	2.5	2.7	2.6	4.0	5
Glutamine	57.7 (46.0–68.2)	0.7	2.0	2.2	1.3	3	0.6	1.3	1.2	1.6	1.8	3.1	5.0	14.6	6
Glycine	32.9 (21.8–46.4)	0.6	0.9	0.4	1.0	0	0.3	0.7	0.6	1.0	0.7	1.1	2.5	4.7	2
Methionine	7.9 (5.4–11.4)	1.1	4.4	6.2	0.7	2		0.8	5.0	0.8	3.5	8.8	9.2	8.5	5
Serine	13.6 (10.3–18.6)	0.8	0.9	0.9	1.9	1	0.9	1.1		1.6	0.9	1.6	4.3	4.2	4
Taurine	16.2 (10.0–25.6)	0.5	0.4	1.3	1.8	1	2.4	0.7		1.0	1.0	4.2	0.5	2.9	2
Tyrosine	14.0 (10.7–18.9)	1.3	2.3	7.1	2.7	3	0.6	1.7	1.1	2.5	1.6	6.4	6.1	5.1	6
Total amino acids increased in each case		2	4	5	5		3	2	2	4	5	8	8	9	

* Expressed as the ratios of values of hepatic coma to average normal values shown in this table. Concentrations of 1.3 times or greater than the normal average for glutamic acid and glutamine, and those of 1.5 times or greater than the normal average are definitely above the normal range, and lower than 0.6 is below the normal range for that amino acid.

† Expressed as micrograms per milliliter of plasma. Values in parentheses indicate ranges of six normal persons used as controls.

the seven amino acids specifically studied, and of taurine and glutamine, were present during hepatic coma, but none appeared to be quantitatively related to the severity of the coma.

MATERIALS AND METHODS

Twelve patients were studied, of whom four were classified as being in a state of precoma; that is, the patients were depressed and coma developed within the next 24 hours, one in light coma and seven in deep coma (Table I). All were observed during the years 1952 and 1953. Blood samples were withdrawn under the conditions summarized in Table I and in the morning after an overnight fast. No patient was receiving amino acid supplements and none received glucose intravenously during the 12 hours prior to withdrawal of blood samples.

Quantities of protein-free, desalted plasma concentrates equivalent to 250 µl. of plasma were used in the determination of free amino acids by two-dimensional paper chromatography using phenol: *m*-cresol and 2,4-lutidine as the solvents (10). The chromatograms were sprayed with an ethanolic ninhydrin solution. Spots of the amino acids to be determined were cut out and the color eluted in a propanol solution. Optical densities of the eluates were measured at 570 mµ. A chromatogram of amino acids serving as the standards was simultaneously prepared. Recovery of amino acids that had been added to the plasma ranged from 90 to 110 per cent.

RESULTS

Table II shows the changes in free amino acids of the plasma during hepatic coma. Glutamic acid, glutamine, and tyrosine increased in three of four patients in precoma; cystine and methionine increased in two patients. Glycine was not increased in any of the four patients in precoma and it actually decreased in one patient. Taurine also decreased in two of these four patients. This probably accounts for the failure to observe a change in total α-amino nitrogen reported earlier (3).

In the single case of light coma, only cystine, glutamic acid, and taurine were significantly increased. Glutamine and tyrosine increased in six of the seven patients in deep coma and cystine, glutamic acid and methionine increased in five of the seven. Glycine and taurine increased in only two patients. Methionine increased in two of four patients in precoma while a similar increase was observed in five of seven patients in deep coma. Methionine was increased more frequently among patients in deep coma than among patients in precoma. In all 12 patients, glutamic acid, glutamine, and tyrosine increased most frequently; cystine and methionine followed in frequency. Glycine increased least frequently.

The concentration of urea in the blood of comatose patients was normal (cases 8 and 9), low (case 3), or high (cases 5 and 11). No relationship between the concentration of urea in the blood and the concentration of free amino acid in the plasma can be discerned from the data. The concentration of amino acids in the plasma of one patient (case 9) who was given four doses of 75 mg. each of cortisone intramuscularly at 4-hour intervals did not change significantly from the concentration in the plasma of patients who were not receiving cortisone.

The chromatogram (Figure 1) shows that the increase of free amino acids in the plasma of a patient (case 11) in deep coma was not limited to the amino acids which were determined quantitatively, but an increase was a general phenomenon. The increase of methionine and α-aminobutyric acid, which has been shown to be formed from methionine (11), was particularly prominent. Although cystine was increased in most patients, taurine was not, which indicated that the formation of taurine from cystine was not increased during hepatic coma.

Our observations on the free amino acids in the urine were similar to those of Walshe (4, 5) in that those patients with greatly increased free amino acids in the plasma also had increased concentrations of amino acids in the urine.

COMMENT

Although we observed some abnormalities in the concentrations of amino acids in the plasma of in-

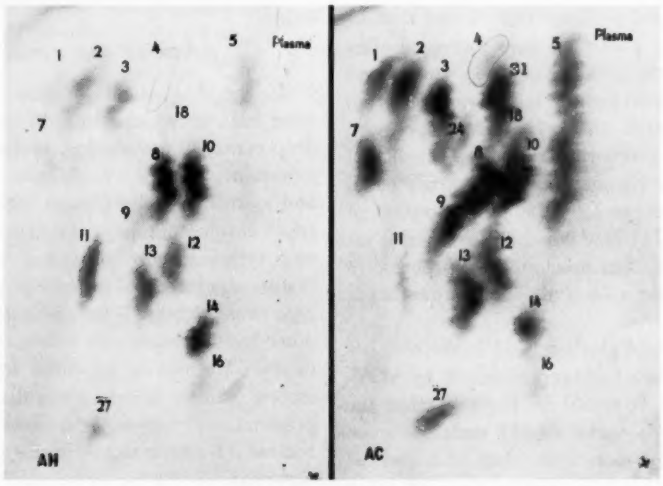

FIG. 1. CHROMATOGRAMS OF FREE AMINO ACIDS OF THE PLASMA OF A NORMAL PERSON (LEFT) AND A PATIENT (CASE 11) IN HEPATIC COMA (RIGHT)

Aliquots corresponding to 300 microliters of plasma of each were applied to sheets at point X. 1, phenylalanine; 2, leucine-isoleucine; 3, valine; 4, proline; 5, arginine-lysine; 7, tyrosine; 8, alanine; 9, threonine; 10, glutamine; 11, taurine; 12, glycine; 13, serine; 14, glutamic acid; 16, aspartic acid; 18, histidine; 24, α-aminobutyric acid; 27, cysteic acid (from cystine); 31, methionine sulfoxide (from methionine).

dividual patients, consistent findings applicable to all patients were not observed. The plasma of a majority of patients in hepatic coma, however, showed increased concentrations of some of the free amino acids. Furthermore, the increases generally were greater among patients in deep coma than among those in precoma. Walshe (4) observed a gross increase in amino acids among patients in hepatic coma. Later, he (5) showed that the urine of most patients having severe hepatic disease contained increased amounts of many amino acids during hepatic coma; the plasma and the spinal fluid also contained increased concentrations of some of the amino acids. In less severe disease, only the urine showed increased concentrations of amino acids. However, no particular amino acid or groups of amino acids appeared to be specifically increased by hepatic disease or by hepatic coma. Increases in several amino acids usually were found but the changes in each specific amino acid varied from patient to patient. On the other hand, Seegmiller, Schwartz, and Davidson (8) reported that there was no increase in the glutamine contained in the plasma of the patient in hepatic coma.

Flock, Mann, and Bollman (12) found that 15 amino acids in the plasma of dogs increased after total removal of the liver. Not every amino acid was always increased and the increase varied from one amino acid to the other. In a later paper with others, they (13) reported that the increase of amino acids in the plasma after hepatectomy could be reduced or prevented by the administration of glucose. They (14) also noted a considerable increase in glutamine but no significant progressive change in glutamic acid of the brain after hepatectomy.

The central role of glutamic acid in the metabolism of brain tissues has been reviewed by Weil-Malherbe (15). It would be expected that the content of glutamic acid should undergo some changes in hepatic coma, if the state of coma was to be related to metabolic disturbances of brain tissues. Our study showed that both glutamic acid and glutamine were increased in a majority of patients during hepatic coma. Walshe (4) suggested that the impaired utilization of glutamic acid may result in failure of intracellular ammonia to be removed from the brain. Although an increase in the concentration of glutamic acid in the plasma was noted, a simultaneous increase in the content of glutamine makes it improbable that the amidation mechanism was impaired. Since the concentration of urea in the blood of many of the patients was normal or high, it is unlikely that the synthesis of urea from ammonia was impaired during hepatic coma.

Our study showed that methionine was significantly increased in a majority of cases. Walshe (4, 5) reported the presence of methionine sulfoxide in the cerebrospinal fluid and plasma during hepatic coma. Since methionine is always spontaneously partially oxidized to its sulfoxide during paper chromatography, it appears that the question of whether the sulfoxide is actually present in the spinal fluid and plasma of a patient in hepatic coma cannot be settled by this analytic procedure. It is noteworthy that the increase of methionine was prominent among patients in the last stages of coma.

The presence of increased amounts of methionine, glutamic acid and other amino acids in the plasma of patients in hepatic coma makes it appear unlikely that further additions of these substances would promote recovery from this condition.

SUMMARY AND CONCLUSIONS

Of the 12 patients observed clinically, four were in precoma, one in light coma, and seven in deep coma. Free alanine, cystine, glutamic acid, glutamine, glycine, methionine, serine, taurine, and tyrosine in the plasma were determined by paper chromatography. Glutamic acid, glutamine, and tyrosine were increased in nine patients; cystine was increased in eight patients, and methionine was increased in seven patients. On the other hand, glycine was increased in only two patients. Methionine appeared to increase oftener among patients in deep coma than among those in precoma. However, consistent changes in the content of amino acids in the plasma were not observed.

REFERENCES

1. Stadie, W. C., and Van Slyke, D. D., The effect of acute yellow atrophy on metabolism and on the composition of the liver. Arch. Int. Med., 1920, **25**, 693.
2. Snell, A. M., and Butt, H. R., Hepatic coma: Observations bearing on its nature and treatment. Tr. A. Am. Physicians, 1941, **56**, 321.

3. Murphy, T. L., Chalmers, T. C., Eckhardt, R. D., and Davidson, C. S., Hepatic coma: Clinical and laboratory observations on forty patients. New England J. Med., 1948, **239**, 605.
4. Walshe, J. M., Observations on the symptomatology and pathogenesis of hepatic coma. Quart. J. Med., 1951, n.s. **20**, 421.
5. Walshe, J. M., Disturbances of aminoacid metabolism following liver injury. Quart. J. Med., 1953, n.s. **22**, 483.
6. Schwartz, R., Phillips, G. B., Gabuzda, G. J., Jr., and Davidson, C. S., Blood ammonia and electrolytes in hepatic coma. J. Lab. & Clin. Med., 1953, **42**, 499.
7. Traeger, H. S., Gabuzda, G. J., Jr., Ballou, A. N., and Davidson, C. S., Blood "ammonia" concentration in liver disease, and liver coma. Metabolism, 1954, **3**, 99.
8. Seegmiller, J. E., Schwartz, R., and Davidson, C. S., The plasma "ammonia" and glutamine content in patients with hepatic coma. J. Clin. Invest., 1954, **33**, 984.
9. Bessman, S. P., Fazekas, J. F., and Bessman, A. N., Uptake of ammonia by the brain in hepatic coma. Proc. Soc. Exper. Biol. & Med., 1954, **85**, 66.
10. Wu, C., Metabolism of free amino acids in fasted and zein-fed rats. J. Biol. Chem., 1954, **207**, 775.
11. Dent, C. E., Methionine metabolism and A-aminobutyric acid. Science, 1947, **105**, 335.
12. Flock, E. V., Mann, F. C., and Bollman, J. L., Free amino acids in plasma and muscle following total removal of the liver. J. Biol. Chem., 1951, **192**, 293.
13. Flock, E. V., Block, M. A., Mann, F. C., Grindlay, J. H., and Bollman, J. L., The effect of glucose on the amino acids of plasma after total hepatectomy. J. Biol. Chem., 1952, **198**, 427.
14. Flock, E. V., Block, M. A., Grindlay, J. H., Mann, F. C., and Bollman, J. L., Changes in free amino acids of brain and muscle after total hepatectomy. J. Biol. Chem., 1953, **200**, 529.
15. Weil-Malherbe, H., Significance of glutamic acid for the metabolism of nervous tissue. Physiol. Rev., 1950, **30**, 549.

STUDIES WITH RADIOACTIVE ENDOTOXIN. I. THE USE OF CR^{51} TO LABEL ENDOTOXIN OF *ESCHERICHIA COLI*[1]

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The endotoxins in different species of gram negative bacteria exhibit remarkably similar biologic effects which have been observed in both experimental animals and man (1–5). These effects may be seen after the injection of living bacteria, killed bacteria, or endotoxin itself; they consist of fever, shock, leukocytosis preceded by leukopenia, hypoglycemia, diarrhea, and prostration. Although there are sometimes hemorrhages in the intestine and other viscera, the findings postmortem are not enough to explain the cause of death or to indicate where the endotoxin acts.

As a means of locating the site of action of endotoxin, as well as its fate *in vivo*, a series of experiments have been carried out using endotoxin labelled with radioactive chromium. The endotoxin of *Escherichia coli* was selected for this study because it can be conveniently prepared in large quantities without the hazards associated with more highly pathogenic bacteria. Radioactive chromium proved to be satisfactory by virtue of its firm attachment to endotoxin, its relative safety in handling, and its half-life of 26.5 days. This report deals with the technique of labelling of *E. coli* endotoxin and with the properties of labelled toxin; those which follow describe its distribution *in vivo* under various experimental conditions.

EXPERIMENTAL METHODS

I. *Preparation of E. coli Endotoxin*

The method of preparation of *E. coli* endotoxin was based on Boivin and Mesrobeanu's (6) principle that endotoxin can be extracted by trichloroacetic acid at cold temperatures. The bacteria were grown in a synthetic medium modified from that described by Gladstone (7) for growth of *S. typhosa*. For the preparation of 25 liters, the following constituents were used: KH_2PO_4 112.5 grams, $(NH_4)_2SO_4$ 12.5 grams, and NH_4Cl 12.5 grams.

These salts were dissolved in 12 liters of distilled water and 700 ml. 1 N NaOH were added, the volume being made up to 15,000 ml. with distilled water. The reaction was adjusted to pH 7.6 by the addition of 1 N NaOH, 9125 ml. distilled water were added, and the medium was put in 4 liter quantities into 6 flasks of 6 liter size and autoclaved at 115° C. for 20 minutes at 15 lbs. pressure. To each flask the following sterile solutions were added: M/6 $MgSO_4 \cdot 7H_2O$ 40 ml., 50 per cent glucose solution 20 ml., and M/$NaHCO_3$ 10 ml. The solutions of $MgSO_4$ and glucose had been sterilized by autoclaving at 115° C. for 15 minutes at 15 lbs. pressure, and the $NaHCO_3$ was sterilized by Seitz filtration.

Care was taken not to add these solutions more than 24 to 48 hr. before the medium was to be used, as storing for longer periods leads to a reduction in the amount of growth (8).

For inoculating this synthetic medium, a strain of *E. coli* was used which had been isolated from a human infection. It was subcultured in 5 per cent rabbit blood broth and on rabbit blood agar to maintain a smooth culture. One or two smooth colonies from the blood agar plate were transferred to 10 ml. of synthetic medium. After 6 to 8 hours' growth at 37° C., the 10 ml. of culture was used to inoculate 400 to 600 ml. of the synthetic medium and this inoculated medium was allowed to incubate overnight at 37° C. Then 50 to 100 ml. portions of the overnight growth were transferred to each of 6 flasks containing 4,000 ml. of synthetic medium.

E. coli grew profusely in this medium. After 24 hours' growth at 37° C., the bacterial bodies were chilled in the deep freeze and removed just as ice began to form. The chilling was necessary to arrest growth until they could be separated in a DeLaval cream separator. If they were not chilled, significant loss of toxicity of both the dried bacterial bodies and the endotoxin occurred as measured by the LD_{50} in mice. By slow addition of the bacterial suspensions to the separator, it was possible to remove the bacteria so effectively that hardly any turbidity remained in the supernatant. The harvested bacteria were removed from the bowl of the separator as a thick moist paste and suspended in enough 95 per cent alcohol to give a final concentration of approximately 70 per cent alcohol. This was followed by two more washings with 95 per cent alcohol, two with acetone, and finally by drying at 37° C. for at least two days.

The dried bacterial bodies were dispersed in 5 times

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their weight of water and shaken with glass beads in a mechanical paint shaker for 15 minutes. The suspension was refrigerated overnight and an equal volume of ice cold .25 M trichloroacetic acid was added the following morning. The centrifuge bottle containing the cold acid suspension was wrapped in towels for insulation, shaken for 15 minutes in the paint shaker and then placed in cracked ice for three hours with frequent shaking by hand. At the end of the period, virtually all of the bacterial residue was removed from the thick suspension by centrifugating in a Spinco ultracentrifuge for 30 minutes at 20,000 r.p.m.; then the slightly opalescent centrifugate was dialyzed in a collodion sac at room temperature against running tap water for 18 hours and then against frequent changes of distilled water for at least 24 hours. The dialysis tubing was next placed in the incubator at 40° C. and the contents concentrated to ½ or ⅓ of the original volume by evaporation with the assistance of an electric fan blowing against the sac. The concentrated solution was passed through a Seitz filter, and the endotoxin was precipitated from the filtrate by the addition of ethyl alcohol to give a final concentration of 68 per cent alcohol by weight. The alcoholic precipitation was allowed to proceed overnight in the refrigerator. The endotoxin thus precipitated, was washed twice with 95 per cent alcohol and twice with ether. It was dried first at 37° C. and then *in vacuo* at 40° C. to a constant weight. The final product, a white powder, was stored in the desiccator with Dririte. The LD_{50} for each batch of dried bacterial bodies and endotoxin was calculated by the method of Reed and Muench (9). Each determination was based on the inoculation of 6 graded doses of endotoxin in 6 groups of mice containing 6 mice per group. Adult white Swiss mice weighing 20 grams were used. The endotoxin reacted negatively to the biuret test for proteins and to the ninhydrin test for proteins, polypeptides, and amino acids.

II. *Tagging E. coli Endotoxin with Radioactive Chromium* (Cr^{51})[2]

E. coli endotoxin has been tagged firmly with hexavalent chromium $Na_2Cr^{51}O_4$ and with trivalent chromium, $Cr^{51}Cl_3$. The endotoxin has been firmly tagged in each of three ways:

A. Adding $Na_2Cr^{51}O_4$ to growing cultures of *E. coli* and extracting the tagged endotoxin.

B. Adding $Na_2Cr^{51}O_4$ to a suspension of the dried bacterial bodies and extracting the tagged endotoxin.

C. Adding $Na_2Cr^{51}O_4$ or $Cr^{51}Cl_3$ to the endotoxin directly. The procedures for accomplishing these tags are as follows:

A. *Adding $Na_2Cr^{51}O_4$ to growing cultures*

Both synthetic and standard media have been used.

1) *Standard media. E. coli* was grown for 48 hours on tryptose agar at 37° C. in modified Blake bottles and washed off with sterile distilled water. The bacterial sediment was washed twice in sterile distilled water and then refrigerated for two days. The bacteria were then suspended in 1,800 ml. of tryptose phosphate broth to which 7.04 mg. of $Na_2Cr^{51}O_4$ (3.24 mc. Cr^{51}) were added. After incubation at 37° C. for 12 hours, the bacteria were again sedimented at 1,500 r.p.m. in the centrifuge and washed twice with sterile distilled water. Successive washing was then performed with 70 per cent alcohol, 95 per cent alcohol (2 washings), acetone and ether. The 7.5 gm. of bacteria obtained in this fashion were dried in a vacuum oven at 40° C.

The total mass of 7.5 gm. of bacteria was suspended in 37.5 ml. of distilled water, shaken in a paint shaker for 30 minutes and cooled to 0° C. in an ice bath. The cold bacterial suspension was mixed with an equal volume (37.5 ml.) of ice cold .25 molar trichloroacetic acid and the endotoxin extracted in the manner described in section I.

The precipitate obtained after treatment with 68 per cent alcohol was washed with 95 per cent ethyl alcohol repeatedly until the count on the washes in the scintillation counter[3] equalled that of the background count. The precipitate was further washed in ether-acetone and dried at 40° C. in the vacuum oven. The total yield of endotoxin was 46 mg.

2) *Synthetic medium.* Eighteen liters of the ammonia medium described above were inoculated with a culture of *E. coli.* After 12 hours' incubation at 37° C., 0.7 mc. of $Na_2Cr^{51}O_4$ (.194 mg.) was added to 9 liters of culture and incubated at 37° C. for an additional 16 hours. After 28 hours, ethyl alcohol was added to a concentration of 20 per cent by weight, all bacteria were separated in the DeLaval cream separator in the manner described, and washed with alcohol, acetone, ether, and dried. The yield of tagged bacteria was 7.2 gm. and of non-tagged bacteria 5.6 gm.

Endotoxin was obtained by extraction with trichloroacetic acid in the cold by the method described above to give 22.9 mg. of tagged endotoxin and 20.3 mg. of non-tagged endotoxin.

B. *Addition of $Na_2Cr^{51}O_4$ to a suspension of the dried bacterial bodies and then extracting the tagged endotoxin*

Modified Blake bottles lined with tryptose agar were inoculated with *E. coli* and incubated at room temperature (27° C.). After 24 hours, the growth was washed off with sterile distilled water. The bacteria were separated from the distilled water in the centrifuge and then washed with 95 per cent ethyl alcohol twice and acetone-ether once. The residue was brought to constant weight by drying at 40° C. in the vacuum oven and storing in a desiccator with calcium chloride. Then 5.3 gm. of dried bacteria were suspended in 100 ml. of distilled water to which were added 4 mc. of $Na_2Cr^{51}O_4$ (1.7 mc.

[2] Radioactive Pharmaceuticals Division, Abbott Laboratories, Oak Ridge, Tennessee.

[3] Radioactivity in all experiments was measured by a gamma sensitive sodium-iodide thallium scintillating well-type crystal.

per mg.). The mixture of bacterial bodies and Cr^{51} was incubated at 37° C. for 48 hours and dialyzed four days against running tap water followed by 24 hours against distilled water to remove free Cr^{51}. The volume was concentrated to 50 ml. *in vacuo* and the suspension chilled to 0° C. The chilled suspension of bacteria was mixed with ice cold 0.25 M trichloroacetic acid, and the endotoxin extracted in the manner described. The yield was 39.5 mg.

To test the stability of the bond between Cr^{51} and endotoxin, the entire volume of tagged endotoxin solution was dialyzed against distilled water at 4° C. for 10 days. Under these circumstances less than 1 per cent of the radioactivity on the endotoxin appeared in the dialysate water during a period of 24 hours.

C. *Adding $Na_2Cr^{51}O_4$ and $Cr^{51}Cl_3$ to the endotoxin directly*

1) *Addition of $Na_2Cr^{51}O_4$ and $Cr^{51}Cl_3$ to non-tagged endotoxin.* *E. coli* endotoxin was prepared from bacteria grown in the synthetic ammonia medium as described above and suspended in water in a concentration of 5 mg. per ml. It was filtered through a Seitz filter to sterilize the endotoxin. The LD_{∞} for mice before and after filtration was .25 mg.

Two equal samples of endotoxin were incubated at 37° C. for 24 hours with Cr^{51} as follows:

a. 7.0 ml. endotoxin (35 mg.) + 0.4 mc. (0.5 ml.) $Cr^{51}Cl_3$

b. 7.0 ml. endotoxin (35 mg.) + 0.4 mc. (0.5 ml.) $NaCr^{51}O_3$

At the end of 24 hours' incubation, each solution was dialyzed against distilled water at room temperature until there was no longer any significant loss of radioactivity into the dialysate water. The results may be summarized as follows:

Source of tag	Mg. of endotoxin	Counts per sec. per mg. Scintillation counter	LD_{50} for mice
Na_2CrO_4	35	593.6	.25 mg.
$CrCl_3$	35	4,771.2	Not lethal

The loss of toxicity was attributed to inactivation of the endotoxin upon exposure to the low pH (pH = 2) of the solution of $CrCl_3$. Precautions were taken, therefore, to prevent inactivation of endotoxin by the acidity of $CrCl_3$. Accordingly, the endotoxin was suspended in a phosphate chloride buffer (pH = 7) before addition of $CrCl_3$. The formula of the buffer was: .3530 g · NaH_2PO_4 + .6390 g Na_2HPO_4 + .172 g NaCl dissolved in 1,000 ml. of water.

Furthermore, to avoid possible loss of endotoxin into the bath during dialysis, in amounts which could not be determined easily, it was decided to rid the endotoxin of free chromium by repeated washes with absolute alcohol in which the endotoxin is insoluble. Two lots of endotoxin were suspended in sterile phosphate chloride buffer and incubated in the presence of Cr^{51} for 24 hours at 37° C. as follows:

Lot A

100 mg. endotoxin plus .56 mg. $Cr^{51}Cl_3$ (.165 mc.)
200 mg. endotoxin plus 1.12 mg. $Na_2Cr^{51}O_4$ (1.057 mc.)
100 mg. endotoxin plus no chromium

Lot B

115 mg. endotoxin plus .77 mg. $Cr^{51}Cl_3$ (.170 mc.)
115 mg. endotoxin plus .505 mg. $Na_2Cr^{51}O_4$ (.355 mc.)
85 mg. endotoxin plus no chromium

After 24 hours' incubation, each sample of endotoxin was reprecipitated by adding ethyl alcohol to a concentration of 68 per cent by weight and separated in the ultracentrifuge at 30,000 r.p.m. for 15 minutes. Each sample was stored in 95 per cent alcohol at 4° C. and then washed repeatedly with absolute alcohol for eight days until no radioactivity appeared in the wash. Then the endotoxin was washed in ether and dried *in vacuo* at 40° C. until it reached constant weight. The radioactivity and LD_{∞} of each sample were measured and listed in Table II (Samples 5–8).

2) *Addition of $Na_2Cr^{51}O_4$ to endotoxin already tagged with chromate ions.* Endotoxin tagged as described in section B above by addition of $Na_2Cr^{51}O_4$ to dead bacterial bodies, was used in this experiment to determine if the tag could be increased by additional exposure. The endotoxin was suspended in water in a concentration of 5 mg. per ml. A total of 10 mg. of tagged endotoxin was incubated for 24 hours at 37° C. with 0.5 mc. of $Na_2Cr^{51}O_4$ and dialyzed against distilled water in parallel with an equal amount of the same batch of tagged endotoxin which had been exposed to no additional $Na_2Cr^{51}O_4$. The dialysis was carried out at 4° C. for 10 days. At that time, no loss of radioactivity was detected in the dialysis bath. The radioactivity of the two samples is given in Table II, number 3A.

III. *Experiments to Provide Evidence that the Tag is Stable and that Radioactivity is Proportional to Toxicity*

This problem was approached in three ways:

1) Labelled endotoxin was dialyzed during storage for as long as one month and the dialysate examined for free Cr^{51} by measuring the radioactivity. In addition, radioactive endotoxin was washed repeatedly in 68 per cent alcohol and the alcoholic wash tested for radioactivity.

2) Suspensions of radioactive labelled endotoxin were subjected to vigorous physical treatment in an effort to rupture the bond. This procedure, consisting of differential ultracentrifugation and alcoholic precipitation, also served as a test for the homogeneity of the material. If the fractions of endotoxin separated by these procedures were of different composition, and if they possessed different affinities for Cr^{51}, it would be expected that the radioactivity per unit of toxicity would vary from fraction to fraction.

3) Serial dilutions of radioactivity were made to learn whether simple dilution of radioactivity was accompanied

by proportional changes in toxicity. This procedure was necessary in order to predict whether the dilution occurring after *in vivo* injection would invalidate radioactivity as a measure of toxin.

The first endotoxin employed was labelled with $Na_2Cr^{51}O_4$. A total of 58 mg., giving 6,064 counts per second in the scintillation counter, was suspended in 23.5 ml. of phosphate buffer. Three fractions of the suspension were separated as follows:

Fraction I was the sediment obtained by centrifugation for one hour at 40,000 r.p.m. in the ultracentrifuge.

Fraction II was obtained by treating the clear supernatant from part I with ethyl alcohol to give a final concentration of 68 per cent by weight, and by ultracentrifugation at 20,000 r.p.m. for 30 minutes. The precipitate was then resuspended in 20 ml. of the phosphate buffer.

Fraction III was the supernatant from part II. It was dialyzed against phosphate buffer for 72 hours after the alcohol had first been removed by evaporation at 37° C. The volume was brought up to 20 ml. with the necessary quantity of phosphate buffer.

Six serial two fold dilutions of each of the three fractions and of the original untreated parent endotoxins were made in phosphate buffer so that the approximate LD_{50} of each fraction could be determined in terms of radioactivity. The radioactivity of each diluted portion was determined and then 0.5 ml. portions of each of the 6 serial dilutions were inoculated intraperitoneally into each of 5 mice. The mortality rate was recorded during 36 hours for the 30 mice receiving each fraction.

The experiment was then repeated using a second endotoxin. This was divided into two portions: one was labelled with $Na_2Cr^{51}O_4$ and the other with $Cr^{51}Cl_3$. A total of 60 mg. of chromate-labelled endotoxin gave 18,540 counts per second in the scintillation counter and a total of 80 mg. of chromic-labelled endotoxin gave 146,560 counts per second. These samples of endotoxin were then treated in the same way as the first endotoxin.

RESULTS

I. *Preparation of endotoxin*

Table I summarizes the results of preparing 9 lots of endotoxin in a synthetic medium.

A comparison of the toxicities of each lot of endotoxin with the toxicity of dried bacterial bodies, from which they were extracted, is expressed as the ratio of their LD_{50}'s. The ratio of LD_{50} of the whole bacterial body to the LD_{50} of purified endotoxin varies in different batches from 9.05 to 2.50. This would suggest that at least 1/2.5 to 1/9 of the dried bacterial cell is composed of endotoxin. The average dried cell would contain at least 1/4.6 (22 per cent) endotoxin according to this type of calculation. It is emphasized that these are minimum values because of the possibility that endotoxin might lose activity during its extraction.

II. *Tagging E. coli endotoxin with radioactive chromium (Cr^{51})*

The results are summarized in Table II. The number of mcg. of atomic chromium bound to each mg. of endotoxin was calculated from its radioactivity, expressed per second. This calculation could be made from the specific activity (mc. per mg.) of the Cr^{51} and the determination of the number of counts per mg. of atomic chromium. The chromium atom constitutes exactly ⅓ of each molecule of $CrCl_3$ and Na_2CrO_4. Each mc. gave 1,650 counts per second in the scintillation counter. All results are corrected for decay.

It is clear that much more Cr^{51} is bound by endotoxin in the presence of $Cr^{51}Cl_3$ than in the presence of $Na_2Cr^{51}O_4$ under conditions in which the relative concentrations of endotoxin and Na_2CrO_4 were the same as those of endotoxin and $CrCl_3$. Under these circumstances, the number of chromate and chromic ions were also approximately the same because the molecular weights of the two compounds were nearly equal. The results also show that much more chromium was bound when $Na_2Cr^{51}O_4$ was incubated with the endotoxin than with the living or dead bacterial cell. In this connection, it was found that the amount of Cr^{51} bound by radioactive endotoxin extracted from dead bacterial cells could be increased by 200 per cent upon subsequent exposure of the extracted endotoxin to $Na_2Cr^{51}O_4$ (Endotoxin 3A, Table II).

The toxicity of the endotoxin as measured by LD_{50} was not changed in any of the experiments by labelling with buffered radioactive chromium. Intravenous injection of 1.0 mg. of the labelled endotoxin was also able to induce the generalized Shwartzman reaction with bilateral cortical necrosis of rabbit kidneys after a preparatory injection of 2.0 ml. of meningococcal filtrate diluted 1:80.[4]

III. *Evidence that the tag is stable and that radioactivity is proportional to toxicity*

The daily loss of radioactivity in the alcoholic washes or in the dialysis wash was less than 0.1

[4] The meningococcal filtrate was obtained from Dr. Richard T. Smith, Department of Pediatrics, University of Minnesota.

TABLE I

Summary of results obtained in preparing endotoxin in a synthetic medium *

A Lot No.	B LD_{50} (mg.) bacterial bodies	C LD_{50} (mg.) endotoxin	D $\frac{LD_{50}\text{ bacterial cell}}{LD_{50}\text{ endotoxin}}$	E Wt. bacterial bodies (gm.)	F Estimated total content of endotoxin (gm.) (E/D)	G Wt. endotoxin produced (mg.)	H % yield of total estimated endotoxin (G/F ×100)
1	1.10	0.375	1.10/0.375 = 2.93	27.7	9.45	952	10.07
2	0.917	0.11	0.917/0.11 = 8.34	7.1	0.85	166.9	19.67
3	0.86	0.095	0.86/0.095 = 9.05	23.7	2.62	713.6	27.27
4	0.312	0.058	0.312/0.058 = 5.37	33.0	6.14	1,184	19.28
5	0.465	0.156	0.465/0.156 = 2.98	25.5	8.55	lost	
6	0.34	0.085	0.34/0.085 = 4.0	18.0	4.50	563	12.51
7	0.167	0.063	0.167/0.063 = 2.65	10.0	3.77	105†	2.78
8	0.625	0.250	0.625/0.250 = 2.50	16.0	6.40	510	7.90
9	0.312	0.094	0.312/0.094 = 3.32	18.0	5.42	280	5.16

* The approximate dilution of endotoxin within the dried bacterial cell can be calculated from the following ratio: $\frac{LD_{50}\text{ of bacterial cell}}{LD_{50}\text{ of endotoxin}}$. Knowing the dilution of endotoxin within the cell, it is possible to calculate the weight of endotoxin in a mass of bacterial bodies from their total weight. It is emphasized that this calculation does not take into account possible loss of toxicity resulting from extraction of endotoxin. For this reason, the estimated content of endotoxin gives only a minimum value. Since the same procedure for extraction was used in all experiments, however, this calculation is valid for comparing the theoretical and total yields in each of the 9 lots. The endotoxin contained .013 mg. nitrogen per gram endotoxin in one of the lots tested. Three other lots contained no nitrogen.

† A portion was lost during processing.

TABLE II

Summary of results obtained in tagging endotoxin *

Culture medium	Point at which Cr^{51} was added	Form of Cr^{51} and no. of mc. added	Mg. Cr^{51} added as atomic chromium	Mg. endotoxin recovered	Counts/sec./mg. endotoxin	μc Cr^{51}/mg. endotoxin	mcg. Cr/mg. endotoxin	LD_{50} for mice (mg. radioactive endotoxin)	Identification No.
Tryptose agar and broth	Growing culture	3.24 Na_2CrO_4	2.34	46	154.4	0.0936	0.0665	0.25	1
Synthetic	Growing culture	0.7 Na_2CrO_4	0.194	22.9	81.6	0.049	0.0136	Not tested	2
Tryptose agar	Dead bacterial bodies	4.0 Na_2CrO_4	0.785	39.5	518	0.387	0.0763	0.25	3
Tryptose agar	Radioactive endotoxin prepared in 3	0.5 Na_2CrO_4	0.298	12	1,552	0.610 (.387 + .223)	.2273 (.0763 + .151)	Not tested	3A
Synthetic	Endotoxin	0.5 Na_2CrO_4	0.174	35	1,187.2	0.719	0.251	0.25	4
Synthetic	Endotoxin	0.165 $CrCl_3$	0.185	58.1	4,205	2.648	2.85	0.071	5
Synthetic	Endotoxin	1.057 Na_2CrO_4	0.369	145.4	973.5	0.59	0.206	0.129	6
Synthetic	Endotoxin	0.170 $CrCl_3$	0.254	98	2,656	1.61	2.40	0.162	7
Synthetic	Endotoxin	0.355 Na_2CrO_4	0.166	109	468.9	0.284	0.137	0.177	8

* Summary of results obtained in labelling endotoxin by adding Cr^{51} before or after separation of endotoxin from bacteria: Efficiency of tag is best expressed as micrograms of chromium bound per mg. of endotoxin (recorded in third column from right). Note that endotoxin binds 10 times as much trivalent as hexavalent chromium. Micrograms of chromium per mg. of endotoxin are calculated from: 1) radioactivity of endotoxin and 2) specific activity (microcuries/microgram) of chromium compound used for labelling that sample of endotoxin. One microcurie produced 1,650 counts per second in the scintillation counter.

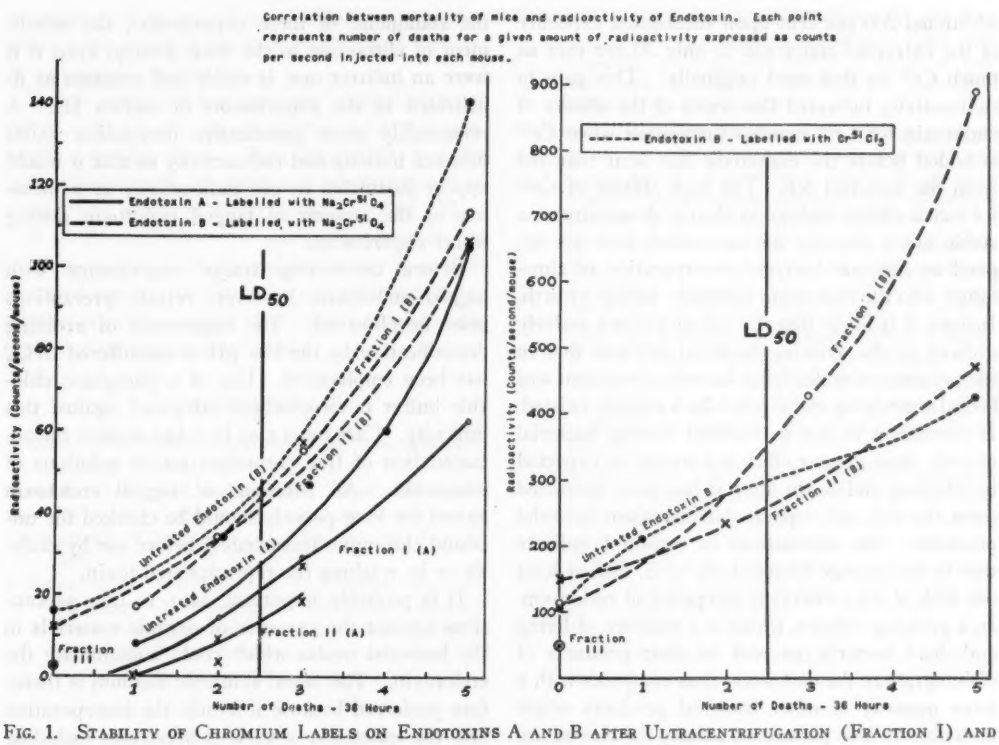
Correlation between mortality of mice and radioactivity of Endotoxin. Each point represents number of deaths for a given amount of radioactivity expressed as counts per second injected into each mouse.
Endotoxin A - Labelled with $Na_2Cr^{51}O_4$
Endotoxin B - Labelled with $Na_2Cr^{51}O_4$
Endotoxin B - Labelled with $Cr^{51}Cl_3$
LD50
Radioactivity (Counts/second/mouse)
Number of Deaths - 36 Hours
Untreated Endotoxin (B)
Untreated Endotoxin (A)
Untreated Endotoxin B
Fraction I (B)
Fraction II (B)
Fraction I (A)
Fraction II (A)
Fraction III

FIG. 1. STABILITY OF CHROMIUM LABELS ON ENDOTOXINS A AND B AFTER ULTRACENTRIFUGATION (FRACTION I) AND ALCOHOLIC REPRECIPITATION (FRACTION II)

Fraction III is the supernate after removal of I and II. Each fraction and the parent endotoxin was divided into 6 serial dilutions and then each dilution inoculated into 5 mice for plotting these mortality curves. All 6 points are not plotted on each curve because only the highest of the dilutions giving 100 per cent mortality is needed. This experiment illustrates not only stability of the tag in the face of vigorous physical manipulation but also a constant ratio between radioactivity and toxicity among the various fractions.

per cent per day for either chromate- or chromic-labelled endotoxin.

In Figure 1, the number of deaths is plotted against the number of counts per second in each sample of endotoxin injected. It is to be noted that for a given endotoxin, the amounts of radioactivity corresponding to the LD_{50} of each the first two fractions were almost identical to that of the original untreated parent endotoxin. Each of the third fractions, consisting of the supernate after sedimentation of endotoxin, contained only slight radioactivity, or none at all, and no toxicity. In other words, radioactivity maintained a constant relationship to toxicity (LD_{50}) despite the manipulations to which endotoxin was subjected. It can also be observed that serial dilutions of the endotoxin produced a proportional fall in toxicity and radioactivity. These facts are good evidence that the endotoxin is a homogeneous substance which binds Cr^{51} tightly and evenly.

DISCUSSION

There can be little doubt, in the case of hexavalent Cr^{51}, that endotoxin is more efficiently labelled after it has been separated from the bacterial cell. This conclusion is supported by two observations: 1) Under conditions which employed an excess of chromate ions, much greater radioactivity per milligram of endotoxin resulted when Cr^{51} was added directly to endotoxin than when it was added to growing cultures; 2) radioactive endotoxin, extracted from a suspension of dead bacterial bodies which had been mixed with Cr^{51} gained an amount of bound chromium equal to an

additional 200 per cent upon subsequent exposure of the extracted endotoxin to only 30 per cent as much Cr^{51} as that used originally. This gain in radioactivity indicated that much of the affinity of endotoxin for Cr^{51} remains unsatisfied when Cr^{51} is added before the endotoxin has been removed from the bacterial cell. The high affinity of Cr^{51} for extracellular endotoxin clearly demonstrates a stable union between the two which does not depend on intimate bacterial incorporation of chromium into the endotoxin molecule during growth. Instead, it is likely that chromium was not actively utilized by the growing bacterial cell and that in the presence of multiplying bacteria chromium was bound entirely by endotoxin which already existed. If chromium is not assimilated during bacterial growth, than greater efficiency would be expected in labelling endotoxin after it has been extracted from the cell and separated from other bacterial products. The calculations in section I indicate that in the average bacterial cell of *E. coli*, at least one-fifth of the contents is composed of endotoxin. In a growing culture, which is a mixture of living and dead bacteria, as well as their products of disintegration, the endotoxin thus competes with a large quantity of other bacterial products which undoubtedly also possess an affinity for chromium. These bacterial products may also combine chemically with the endotoxin itself and thus interfere with its union with Cr^{51}. The number of chromate ions was calculated to be present in great excess of the estimated available endotoxin in all experiments. The presence, however, of other bacterial products capable of binding chromium could appreciably reduce the ratio of free chromate ion to endotoxin molecules.

Both chromate and chromic ions attach themselves to the endotoxin molecule without altering toxicity. Boivin and Mesrobeanu (6) have demonstrated that endotoxin obtained by their method is free of protein and is dissociated into a toxic phospholipid fraction, and a non-toxic polysaccharide fraction. The endotoxin prepared in the present experiments from *E. coli* was also found to be free of protein, polypeptides, and amino acids by the extremely sensitive ninhydrin test. It is possible, therefore, that if chromic ion became attached to the polysaccharide fraction, the chromium would be connected to the toxic moiety through a non-toxic link. Fortunately, from the standpoint of these experiments, the attachment of chromium to the toxic moiety, even if it were an indirect one, is stable and constant as illustrated in the experiments in section III. A remarkably close quantitative correlation exists between toxicity and radioactivity so that it would appear justifiable to use radioactivity as a measure of the amount of tagged endotoxin during tracer experiments.

Before conducting tracer experiments with tagged endotoxin, however, certain precautions must be observed. The importance of avoiding detoxification by the low pH of unbuffered $CrCl_3$ has been emphasized. Use of a phosphate chloride buffer is an excellent safeguard against this difficulty. Care must also be taken against the accumulation of free chromium ion in solutions of endotoxin. All solutions of tagged endotoxin stored for long periods should be checked for unbound chromium immediately before use by dialysis or by washing the reprecipitated toxin.

It is probably important, also, to take precautions against the presence of organic materials in the bacterial media which could contaminate the endotoxin. The liquid synthetic medium is therefore preferred because it avoids the incorporation into the endotoxin of various impurities including the galactose present in agar (8).

SUMMARY AND CONCLUSIONS

E. coli endotoxin has been prepared in a synthetic medium consisting of inorganic salts and dextrose. Dried bacterial cells obtained after growth in this medium were calculated to contain an approximate average of at least 22 per cent endotoxin.

E. coli endotoxin has been firmly labelled with hexavalent Cr^{51} by the addition of $Na_2Cr^{51}O_4$ to suspensions of each of the following:

1) actively growing cultures of *E. coli*

2) dead bacterial cells harvested from the culture medium

3) endotoxin after separation from the bacterial cell.

The most efficient means of labelling with Cr^{51} is by addition to endotoxin after separation from the bacterial cell. This provides a high count per mg. endotoxin with a minimum waste of free

chromium. The tag is firm and the LD_{50} of radioactive toxin is the same as corresponding samples of non-tagged endotoxin.

E. coli endotoxin has also been labelled with trivalent Cr^{51} by the use of $Cr^{51}Cl_3$. $Cr^{51}Cl_3$ produced a far heavier tag than $Na_2Cr^{51}O_4$ and did not reduce toxicity of endotoxin when precautions were taken to prevent inactivation by low pH.

A close quantitative relationship has been shown to exist between toxicity and radioactivity so that it would appear justifiable to use radioactivity as a measure of the amount of chromate tagged endotoxin during tracer experiments.

REFERENCES

1. Petrie, G. F., The endotoxin of the meningococcus. J. Hyg., 1937, **37**, 42.
2. Spink, W. W., and Anderson, D., Experimental studies on the significance of endotoxin in the pathogenesis of brucellosis. J. Clin. Invest., 1953, **33**, 540.
3. Brues, A. M., and Shear, M. J., Chemical treatment of tumors. X. Reactions of four patients with advanced malignant tumors to injection of a polysaccharide from *Serratia marcescens* culture filtrate. J. Nat. Cancer Inst., 1944, **5**, 195.
4. Braude, A. I., Siemienski, J., Williams, D., and Sanford, J. P., Overwhelming bacteremic shock produced by gram-negative bacilli; a report of four cases with one recovery. Univ. Mich. Med. Bull., 1953, **19**, 23.
5. Favorite, G. O., and Morgan, H. R., Effects produced by the intravenous injection in man of a toxic antigenic material derived from *Eberthella typhosa:* Clinical, hematological, chemical, and serological studies. J. Clin. Invest., 1942, **21**, 589.
6. Boivin, A., and Mesrobeanu, L., Recherches sur les antigènes somatiques et sur les endotoxines des bactéries: I. Considérations générales et exposé des techniques utilisées. Rev. d'immunol., 1935, **1**, 553. II. L'antigène somatique complet (antigen O) de certaines bacteries est le constituant principal de leur endotoxine. Rev. d'immunol., 1936, **2**, 113. III. Antigène somatique O "complet" et variations bacteriennes. Rev. d'immunol., 1937, **3**, 319.
7. Gladstone, G. P., The antigenic composition and virulence of *Bact. typhosum* grown on a chemically-defined medium. Brit. J. Exper. Path., 1937, **18**, 67.
8. Freeman, G. G., Challinor, S. W., and Wilson, J., The use of a synthetic medium in the isolation of the somatic antigens of *Bact. typhi-murium* and *Bact. typhosum.* Biochem. J., 1940, **34**, 307.
9. Reed, L. J., and Muench, H., A simple method of estimating fifty per cent endpoints. Am. J. Hyg., 1938, **27**, 493.

STUDIES WITH RADIOACTIVE ENDOTOXIN. II. CORRELATION OF PHYSIOLOGIC EFFECTS WITH DISTRIBUTION OF RADIOACTIVITY IN RABBITS INJECTED WITH LETHAL DOSES OF *E. COLI* ENDOTOXIN LABELLED WITH RADIOACTIVE SODIUM CHROMATE [1]

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The development of a simple technique for labelling *E. coli* endotoxin with radioactive chromium has presented the opportunity for tracer experiments (1). Such experiments have been conducted in different species of animals, using various doses of endotoxin tagged with either hexavalent chromium as $Na_2Cr^{51}O_4$, or trivalent chromium, as $Cr^{51}Cl_3$. One of the objectives was to observe the physiologic changes which unfold as the endotoxin is removed from the blood and deposited in various organs. This paper presents the first of a series of studies describing these changes; it deals with the injection into rabbits of lethal doses of endotoxin tagged with hexavalent radiochromium.

METHODS

Endotoxin, prepared from bacteria grown in synthetic medium, was labelled by direct incubation with $Na_2Cr^{51}O_4$ by the method described in the preceding paper (1). When injected into rabbits, there were 223.4 counts per second per mg. and the LD_{50} for mice before and after tagging was 0.2 mg.

Three groups of adult rabbits weighing 2.5 to 3.0 kg. were injected intravenously with Cr^{51} as follows:

Group I—Endotoxin labelled with $Na_2Cr^{51}O_4$.

Group II—$Na_2Cr^{51}O_4$ in one vein followed by the injection of unlabelled endotoxin in another vein.

Group III—$Na_2Cr^{51}O_4$ only (no endotoxin).

The dose of endotoxin and chromium is given for each group in Tables IA, IB, IC and Figures 1A, 1B, 1C. The dose of radioactive endotoxin was selected after determining from preliminary experiments: 1) that the pattern of distribution of radioactivity was the same over a wide range of lethal doses, and 2) that the amount of radioactive endotoxin injected in these experiments was enough to provide accurate counts in the tissues without killing the animals in too short a time to complete the experiment.

Before the injection of endotoxin, leukocyte counts were made on blood from the ear vein, and rectal temperatures and blood pressures were measured repeatedly until the rabbits were stabilized. The blood pressures were measured by placing a human blood pressure cuff around the abdomen, inflating it in the usual manner, and palpating the femoral arterial pulse until it was obliterated. This method for measuring blood pressure in rabbits has been checked by simultaneous determinations with the ear-chamber apparatus of Grant and Rothschild (2), and the results were identical when the chamber and cuff were connected to the same manometer. Although the abdominal-cuff method may not be as precise as direct intra-arterial measurement, it provides a simple and satisfactory non-surgical technique for demonstrating changes in pressure, and does not require anesthesia which might complicate the studies on endotoxin.

When the animals were about to be sacrificed, determinations of blood pressure, temperature, and leukocyte count were repeated, and 30 to 50 ml. of blood was drawn from the heart. The blood was citrated with one-fourth its volume of ACD (1.32 gm. sodium citrate, 0.48 gm. citric acid, and 1.47 gm. dextrose per 100 ml.) solution and separated into its three components; plasma, red cells, and buffy coat. The plasma was easily removed after centrifugation at 5,000 r.p.m. in the refrigerated ultracentrifuge for 20 minutes. Buffy coat was next removed in the form of a continuous membrane with a Pasteur pipette. The buffy coat and accompanying red cells were suspended in saline in a small test tube (75 × 9 mm.) and centrifuged at approximately 2,000 r.p.m. for 10 minutes. After several washings with saline, the buffy coat had been freed almost entirely of red cells.

Animals were sacrificed by exsanguination, during which process saline was injected intravenously so that the animals remained alive long enough to permit removal of maximum amounts of blood.

The tissues of all sacrificed animals were weighed and then soaked in tap water at 4° C. until the washings contained no appreciable hemoglobin in a 24-hour period. This procedure was necessary to insure that the presence of radioactivity in an organ was not that of the blood contained within it. Admittedly, endotoxin could be lost from the tissues but it is unlikely that a large proportion would disappear in view of the finding that the total radioactivity in the combined washings did not exceed by more than 5 per cent of the total injected that calculated to be present in the blood which had been washed

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TABLE IA

GROUP I

Concentration of Radioactivity in Various Tissues = $\frac{\%\ \text{TOTAL INJECTED ACTIVITY}}{\%\ \text{TOTAL BODY WEIGHT}}$

Time after injection (minutes)	0–15		45		85		105			140	225	240
Rabbit No.	27	28	35	36	32	30	25	31	26	29	33	34
BLOOD:	0	0		.11	0	0	.1	0		1.1		1.1
				.32			.34			.63	0	.74
Red Cells				.30			.3			1.7		1.4
	23.6	23.8		6.13	9.2	7.86	6.11	8.6		12.3	3.3	
	1.86	1.77		.85	.7	1.12	.95	1.07		1.36	.7	
Plasma	12.69	13.46		7.21	13.1	7	6.15	8		9	4.7	
	2.87	1.32		2.69	2.76		2.98	.6		2.54	2.77	2.81
	.00042	.0012		.0021	.00118		.00025	.003		.00048	.003	.004
Buffy Coat	7000	1200		12,000	25,000		18,000	200		6,000	10,000	6,000
	9.22	9.89	17.5	16.26	19.8	14.08	11.79	15.6	17	17.03		17.02
	3.32	2.55	3.6	3.29	2.87	2.5	2.8	3.8	3.37	3.72		3.28
LIVER	2.77	3.87	4.99	4.94	5.11	5.6	4.18	4.1	5.05	4.56		5.19
	2.85	4.87	1.9	2.14	5.3	2.6	6.99	2.95	2.2	2.2	1.42	.23
	.55	.47	4.2	.49	4.7	6	.77	6.4	.8	.59	.48	.34
LUNG	5.18	9.51	4.5	4.38	11.3	4.3	9.07	4.6	2.75	3.76	2.95	0.6
	.26	.17	.42	.37	.05	.22	.37	.36	.36	.18	.28	.94
	.27	.24	.25	.28	.22	.24	.28	.28	.40	.37	.35	.87
HEART	.96	.70	1.68	1.32	.22	.91	1.32	1.2	.9	.49	0.8	1.1
	.85		.8	1.16	1.37	.33	3.18	.77	2.59	.92		
	.66		.65	.73	.71	.52	.63	.74	.64	.61		
KIDNEY	1.28		1.23	1.58	1.9	.6	5.04	1.04	4.05	1.5		
	.22	.15		.31	.31		.21	.2	.72	.2	.34	
	.07	.06		.04	.04		.06	.06	.09	.06	.05	
SPLEEN	3.14	2.5		7.75	7.75		3.5	3.3	8	3.3	6.8	
	1.87	1.15	1.47	4.2		0		1.3	3.2		1.47	1.48
	3.64	4.7	5.31	4.9				2.8	4.64		6.68	6.45
INTESTINE	.51	.24	.27	.86				0.5	.68		0.2	.22
	2.35	1.15		.73	.73			0		1.09	0	1.32
	5.46	4.53		4.98	4					5.20		4.62
MUSCLE	.43	.25		.14	.18					.2		.29
		.56		.86		.79	1.27	.73	1.19	.91	.96	.96
		.43		.56		.59	.67	.55	.48	.43	.75	.45
BONE		1.3		1.53		1.46	1.89	1.32	2.48	2.1	1.3	2.13

Each rabbit received 6 mg. endotoxin containing 223.4 counts per second per mg.

out. After washing, tissues were dried in an oven at 100° C. The muscle and intestine were incinerated to ashes at 500° C. and the liver, lung, heart, kidney, and spleen were digested with concentrated nitric acid. The femurs were dried and pulverized. Plasma, red cells, and buffy coats were weighed but not digested. Total weights per animal of plasma, red cells, and buffy coats were calculated from the weights of the samples examined on the basis of blood volume determinations made in normal rabbits of equivalent size as described below.

The radioactivity of the tissues and injected chromium was determined as previously described in the scintillation counter after counting for 10 minutes (1). Loss of activity resulting from lapse of time during treatment of tissues was accounted for by plotting the time-decay curve.

Observations were also made on blood volume following the injection of endotoxin. Because the method for measuring blood volume was that of tagging red cells with Cr^{51}, it was necessary to inject non-labelled endotoxin. Blood volume studies were conducted in three rabbits receiving endotoxin. Their red cells were obtained for labelling by drawing 20 ml. of heart blood from each into 5 ml. of ACD solution. The red cells were separated by centrifugation at 2,000 r.p.m. for 15 minutes and then the plasma supernate was removed. To

each sample of red cells were added 200 microcuries in .103 mg. of $Na_2Cr^{51}O_4$. The mixture of red cells and chromium was incubated at 37° C. for one hour with frequent gentle agitation. Then the red cells were washed with saline three times, resuspended in their own plasma, and injected back into the rabbit from which they had been removed. The process required a total of 4 to 5 hours. The tagged cells were allowed to circulate for 72 hours before endotoxin was injected because the animals were unduly susceptible to endotoxin if it was injected within a few hours after the tagged cells had been returned to the rabbits.

To determine blood volumes, 4.0 ml. of blood were removed from the animal's heart and the activity determined in a well-type gamma ray counter manufactured by the Texas Company. Blood volume equalled $\frac{\text{total counts injected}}{\text{counts per sec. per ml. of circulating blood}}$. After each rabbit's normal blood volume was determined, the rabbit was given intravenously 1.0 mg. of non-labelled endotoxin. Blood samples of 4.0 ml. were removed from the heart at various intervals up to the time of death and blood pressures were determined periodically. Hematocrits were measured on the 4.0 ml. of cardiac heparinized blood, and leukocytes were counted in the blood from the ear vein. Red cell mass was calculated by multiplying hematocrit times blood volume and corrections were made for those cells removed at the time of each blood volume determination.

To test the distribution *in vitro* of free chromium and

TABLE IB

GROUP II

Concentration of Radioactivity in Various Tissues = $\frac{\%\ \text{TOTAL INJECTED ACTIVITY}}{\%\ \text{TOTAL BODY WEIGHT}}$

Time after Injection (MINUTES)	0-10			40		60	75	90		120	150	330	
Rabbit No.	1	2	3	6	13	7	8	9	5	10	4	11	12
BLOOD:	5.33	3.88		8.43	5.50	3.24			6.3	3.95	3.1	4.6	2.5
	0.3	0.43		0.46	0.34	0.41			0.49	0.26	0.41	0.44	0.14
Red Cells	16.2	9.02		18.3	15.94	7.9			12.8	15.2	7.56	10:45	17.8
	2.83	3.19		1.78	1.78	1.94			1.98	0.91	1.7	0.71	0.35
	1.12	1.73		0.87	0.79	0.83			0.86	0.60	0.64	0.56	0.28
Plasma	2.5	1.84		2.04	2.25	2.33			2.25	1.52	2.65	1.26	1.25
	8.96	7.3	6.3	4.98	4.31	6.21	5.81	5.31	4.6	6.37	5.3	5.4	5.3
	3.1	2.79	3.09	2.55	3.98	2.61	2.15	3.23	3.45	3.22	2.04	2.36	2.14
LIVER	2.89	2.6	2.03	1.95	1.13	2.37	2.47	1.64	1.3	1.95	2.59	2.28	1.94
		1.03	0.43	0.67	0.87	0.87	0.85	0.89	1.10	0.67	0.88	0.72	0.62
		0.78	0.65	0.67	0.93	0.53	1.06	0.67	0.61	0.59	0.94	0.35	0.62
LUNG		1.32	0.66	1.0	1.56	1.64	0.80	1.32	1.80	1.13	1.62	2.18	1.0
	0.49	0.31	0.67	0.26	0.25	0.19	0.22	0.33	0.45	0.36	0.4	0.26	0.18
	0.23	0.32	0.30	0.31	0.27	0.30	0.41	0.40	0.94	0.39	0.26	0.35	0.29
HEART	2.13	0.97	2.23	0.83	.96	0.63	0.53	0.8	1.3	0.92	1.5	0.74	0.62
	3.08	3.5	1.98	6.49	4.98	9.31	6.5	6.89	9.95		8.1	9.49	10.5
	0.62	0.76	0.59	0.72	0.59	0.61	0.94	0.62	0.73		0.51	0.63	0.56
KIDNEY	4.9	4.6	3.35	9.01	8.44	15.5	12.0	11.1	13.63		15.88	15.06	18.7
		0.12	0.07	0.10	0.12	2.95		0.10	.07	0.25	0.73	1.28	.07
		0.05	0.04	0.03	0.08	3.96		0.08	0.04	.05	0.06	0.04	0.05
SPLEEN		2.4	1.75	3.33	1.5	0.7		1.25	1.75	5.0	12.3	32.	1.42
	2.57			2.21	1.10	0.52	1.5			2.41		0.35	1.12
	4.74			4.77	3.02	5.54	4.78			4.48		1.84	4.13
INTESTINE	0.54			0.46	0.36	0.96	0.31			0.53		0.19	0.27
	2.21		2.57	1.03	2.21	2.55	1.10			1.66		1.39	1.10
	5.65		4.20	5.74	6.29	0.78	5.63			5.03		5.66	5.04
MUSCLE	0.39		0.61	0.17	0.35	3.26	0.19			0.33		0.24	0.22
	0.71		0.74	1.19	1.01		0.87	1.35		0.74	0.9	0.56	0.68
	0.77		0.66	0.71	0.48		0.54	0.5		0.62	0.46	0.49	0.47
BONE	0.92		1.12	1.67	2.08		1.61	2.7		1.19	2.0	1.14	1.45

Each rabbit received 5mg. endotoxin in one vein and 2ml. of Na_2CrO_4 solution containing 2506 counts/second/ml. in another vein.

TABLE IC

GROUP III

Concentration of Radioactivity in Various Tissues = $\frac{\%\ \text{TOTAL INJECTED ACTIVITY}}{\%\ \text{TOTAL BODY WEIGHT}}$

Time after Injection (MINUTES)	0-5		60			90		140		369	370
Rabbit No.	14	15	16	20	21	17	22	18	19	23	24
BLOOD:	2.78		5.03	7	4.32	5.76	5.76	5.41	5.41	1.41	6.49
	.21		.84	0.94	.6	0.88	0.77	.77	.94	.14	.82
Red Cells	13.3		5.98	7.44	7.2	6.54	7.48	7.02	5.75	10.07	7.91
			3.95	2.06	3.06		4.32	2.87	1.62	1.78	1.78
			1.41	1.66	1.6		1.81	1.77	1.80	1.86	1.53
Plasma			2.80	1.24	1.9		2.38	1.62	0.90	.95	1.16
	4.63	3.24	6.63	5.31		3.31	4.03	4.64	8.96	5.31	9.95
	3.26	2.35	2.22	3.41		3.44	2.77	2.45	2.16	3.02	2.49
LIVER	1.42	1.37	2.98	1.55		.96	1.45	1.89	4.14	1.75	3.99
	1.42	.98	1.27	0.88		1.42	1.06	0.95	.92		.85
	.68	.48	0.57	0.77		.60	.61	0.52	.41		.62
LUNG	2.08	2.04	2.22	1.14		2.36	1.73	1.82	2.24		1.37
	.31	.22	.34	0.32		.31	.50	.36	0.42	.28	.42
	.27	.23	.23	0.36		.29	.29	.31	.30	.35	.32
HEART	1.14	.95	1.47	.88		1.06	1.72	1.16	1.40	.80	1.31
	1.19		8.32	12.8		9.5	6.36	9.69	.12	4.73	1.71
	.76		0.55	.89		.90	0.72	0.60	.04	35	.30
KIDNEY	1.56		15.1	14.38		10.5	8.8	16.5	3	5.56	5.7 (1 KIDNEY)
	.25	.07	0.10	.09		.22	.09	0.09		.12	.48
	.17	.03	0.02	.07		.08	.08	.10		.05	.04
SPLEEN	1.47	2.33	5	1.28		2.75	1.12	.90		2.40	12
				2.57		3.51	1.59				
				6.42		5.36	5.35				
INTESTINE				0.40		.65	0.29				
			1.42	1.38			1.69				
			4.33	6.18			6.45				
MUSCLE			0.32	.22			0.26				
	1.17	.70	.79	.92		1.55	1.47	1.03		1.08	
	.8	.48	.53	.53		.82	0.83	.60		.64	
BONE	1.46	1.45	1.49	1.73		1.89	1.77	1.71		1.68	

Each rabbit received intravenously 2 ml. of Na_2CrO_4 containing 5012 counts/second/total

chromium bound to endotoxin each was mixed with citrated blood pooled from three rabbits as follows and incubated for one hour at 37° C.:

1.5 ml. $Na_2Cr^{51}O_4$ containing 253 counts per second (Texas well counter) plus 105 ml. pooled blood.

1.5 ml. labelled endotoxin (9 mg.) containing 253 counts per second plus 105 ml. pooled blood.

At the end of the incubation period, the bloods were separated into plasma, red cells, and buffy coat. Then each was examined for radioactivity. Cross matching was performed before the bloods were pooled to be certain that the experiment was not complicated by incompatibilities which might agglutinate red cells, or coat them with antibody in such a way that their affinity for chromium would be altered. Incompatibilities between blood from rabbits have been repeatedly demonstrated in this laboratory.

RESULTS

The results are summarized in Tables IA, IB, and IC, and the corresponding Figures, 1A, 1B, and 1C. It is clear that the distribution of radioactivity after injection of free chromium is entirely different from that after injection of tagged endotoxin. The most remarkable difference concerns the blood. Free chromium passes mainly to the red cells and only small amounts are in the plasma. Chromium attached to endotoxin, on the other

hand, appears in the plasma in large quantities and virtually none goes to red cells. A striking amount of radioactivity appears in the buffy coat after injection of labelled endotoxin.

Among animals given labelled endotoxin, most of the activity in the tissues is found in the liver and only a small per cent in the kidneys. Animals receiving free chromium, by contrast, show heavy activity in the kidneys and urine, but relatively little in the liver. Rabbits receiving labelled endotoxin also show a greater uptake in the lung and spleen than those receiving free chromium. The increased uptake in spleen and lung is shown in Table I which expresses the degree of concentration in tissues as well as per cent uptake. The relatively small size of certain viscera could permit marked concentration even though only a small per cent of total injected activity appears in them. Examinations of brain revealed no radioactivity after injection of labelled endotoxin.

The affinity of free Cr^{51} for red cells is also seen when $Na_2Cr^{51}O_4$ is added to whole blood *in vitro*. Similarly, the failure of labelled endotoxin to appear in red cells is observed when radioactive endotoxin is mixed with whole blood. This is illustrated in the following results expressed as

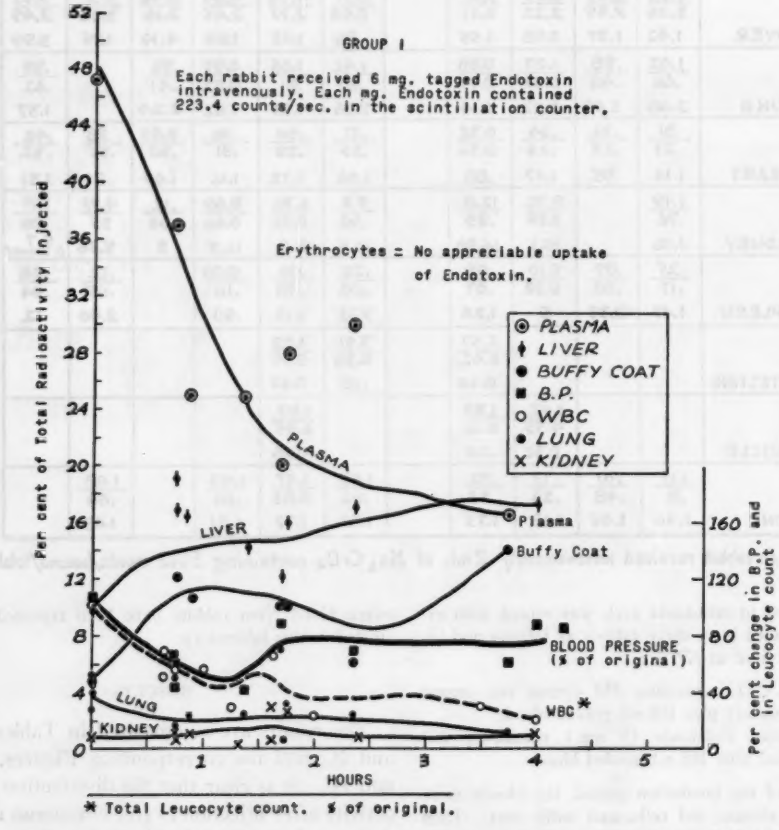

FIG. 1A. DISTRIBUTION OF RADIOACTIVITY AMONG BLOOD AND TISSUES OF RABBITS INJECTED WITH RADIOACTIVE ENDOTOXIN

The leukocyte count and blood pressure at time of sacrifice is expressed as per cent of the control value obtained in the same animal before endotoxin was injected. Each point represents a determination on one sacrificed rabbit.

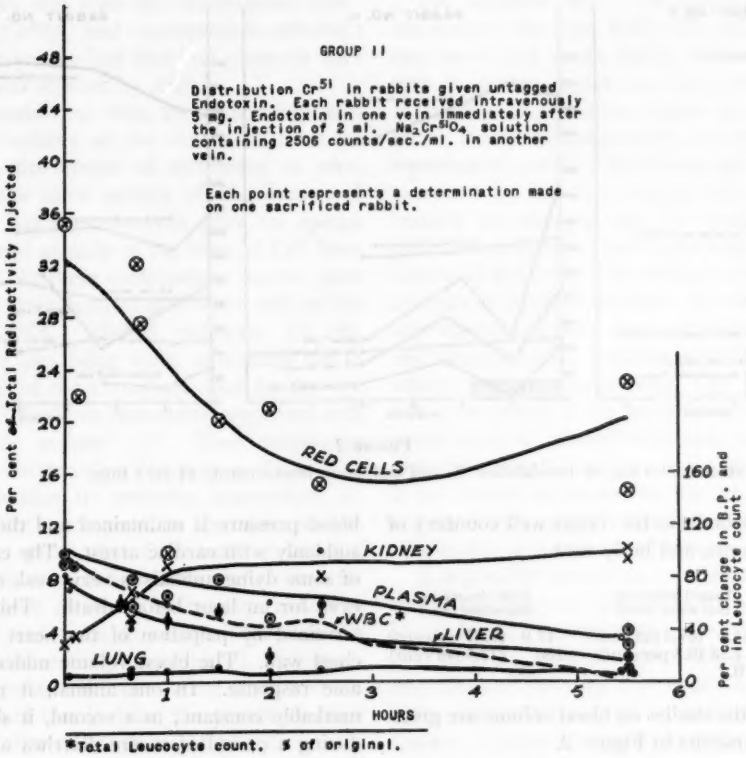
GROUP II
Distribution Cr^{51} in rabbits given untagged Endotoxin. Each rabbit received intravenously 5 mg. Endotoxin in one vein immediately after the injection of 2 ml. $Na_2Cr^{51}O_4$ solution containing 2506 counts/sec./ml. in another vein.
Each point represents a determination made on one sacrificed rabbit.
Per cent of Total Radioactivity Injected
Per cent change in B.P. and in Leucocyte count
RED CELLS
KIDNEY
PLASMA
WBC*
LIVER
LUNG
HOURS
*Total Leucocyte count. % of original.

FIGURE 1B

Symbols same as in 1A.

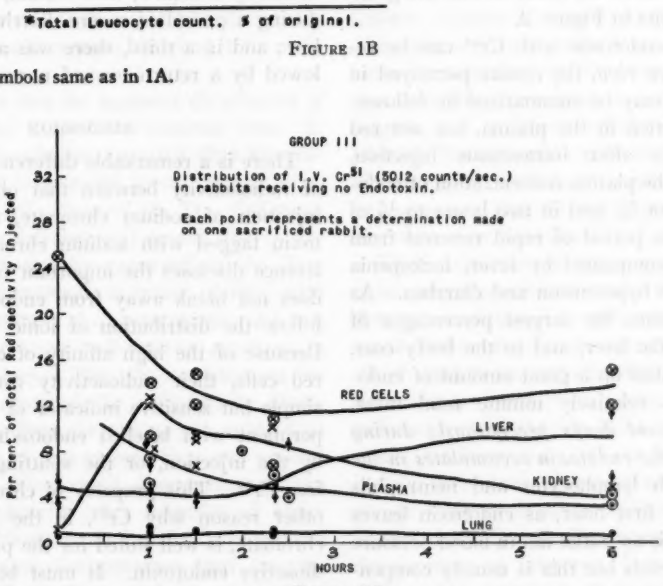

FIGURE 1C

Symbols same as in 1A.

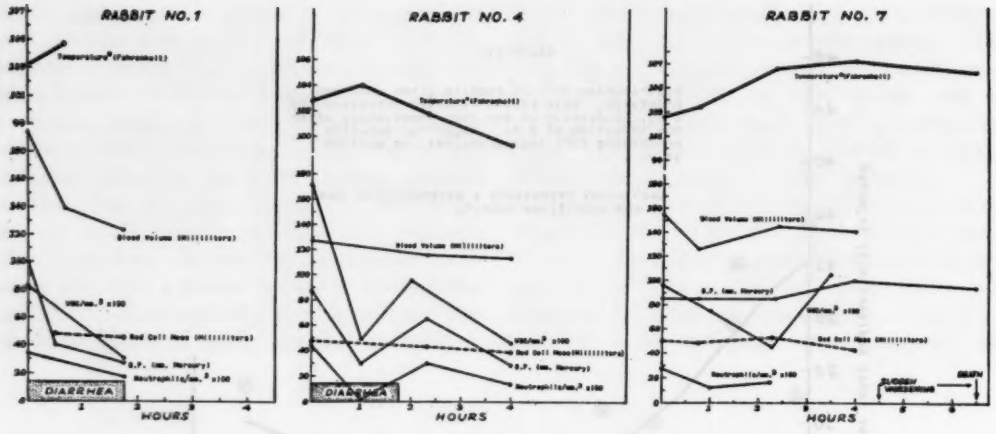

FIGURE 2

Each rabbit received 1.0 mg. of non-labelled *E. coli* endotoxin intravenously at zero time.

counts per second (in the Texas well counter) of plasma, red cells, and buffy coat.

	Tagged endotoxin plus whole blood	Free chromium plus whole blood
Plasma	224.8 (87.3 per cent)	17.9 (7.07 per cent)
Red cells	1.28 (0.5 per cent)	186 (73.5 per cent)
Buffy coat	0.5	.06

Results of the studies on blood volume are given for the three rabbits in Figure 2.

If the tag on endotoxin with Cr^{51} can be regarded as stable *in vivo,* the results portrayed in Figures 1 and 2 may be summarized as follows: Endotoxin is carried in the plasma, but not red cells, immediately after intravenous injection. Within an hour, the plasma concentration of endotoxin falls to about ⅗, and in two hours to ½ of the original. The period of rapid removal from the plasma is accompanied by fever, leukopenia and sometimes by hypotension and diarrhea. As it leaves the plasma, the largest percentages of endotoxin go to the liver, and to the buffy coat. The buffy coat takes up a great amount of endotoxin despite its relatively minute total mass. *The leukocyte count drops precipitously during the period when the endotoxin accumulates in the buffy coat.* Both lymphocytes and neutrophils fall. During the first hour, as endotoxin leaves the plasma, there is an initial fall in blood pressure to nearly shock levels but this is usually compensated for, at least temporarily. Then it may fall again just before death. In other animals the blood pressure is maintained and the animal dies suddenly with cardiac arrest. The cardiac action of some dying animals is very weak and irregular even for an hour before death. This is best determined by palpation of the heart through the chest wall. The blood volume undergoes a variable response. In one animal, it remained remarkably constant; in a second, it showed a fall during a period of severe diarrhea and remained low; and in a third, there was an initial fall followed by a return toward normal in two hours.

DISCUSSION

There is a remarkable difference in distribution of radioactivity between that obtained after the injection of sodium chromate, and after endotoxin tagged with sodium chromate. This difference discloses the important fact that the Cr^{51} does not break away from endotoxin *in vivo* to follow the distribution of ionic hexavalent Cr^{51}. Because of the high affinity of chromate ion for red cells, their radioactivity can be used as a simple but sensitive indicator of whether the experiment with labelled endotoxin is complicated by the injection, or the splitting-off *in vivo,* of free Cr^{51}. This property of chromate ion is another reason why Cr^{51}, in the form of sodium chromate, is well suited for the preparation of radioactive endotoxin. It must be emphasized, in this connection, that endotoxin does not reduce affinity of red cells for chromate ion. The radio-

activity of red cells after the simultaneous injection of $Na_2Cr^{51}O_4$ and non-labelled endotoxin (Figure 1B) was no less than that observed after $Na_2Cr^{51}O_4$ was injected by itself.

Other experiments from this laboratory also support the validity of the chromium label for tracing the distribution of endotoxin *in vivo*. Essentially the same pattern of distribution of radioactivity has been observed when the species of experimental animals or the form of Cr^{51} have been varied. In these confirmatory studies, mice received chromate labelled endotoxin and rabbits chromic ($Cr^{51}Cl_3$) labelled endotoxin (3, 4). Attempts are also being made to confirm the *in vivo* stability of the chromium label by the immunochemical methods customarily employed with simple protein antigens (5). These techniques are of questionable value, however, in the case of endotoxin because its antigenic components do not necessarily correspond to its toxic components. Final proof that Cr^{51} remains attached *in vivo* to the toxic portion of the endotoxin complex will depend on recovery from the tissues of Cr^{51}-labelled endotoxin in such a state that the amount of radioactivity per unit of toxicity is equal to or greater than that of the inoculated endotoxin. Until this is done, the possibility must be conceded that endotoxin could be split *in vivo* and that Cr^{51} could remain attached to a nontoxic portion.

It is significant that the apparent distribution of chromium-labelled endotoxin obtained from *E. coli* resembles closely that reported after inoculation of iodinated endotoxins from *Serratia marcescens* and *Shigella flexneri* (6, 7). Seligman, Shear, Leiter, and Sweet (6) found that by far the greatest radioactivity appeared in the liver soon after the intravenous injection of mice with I^{131}-labelled endotoxin of *S. marcescens;* and that next to the liver, most radioactivity appeared in the lung and kidney. They did not examine the buffy coat, but reported that radioactivity disappeared so rapidly from the plasma of a rabbit that only 24 per cent of that at zero time was present at four hours. This rapid disappearance from plasma of radioactivity after injection of endotoxin labelled with either Cr^{51} or I^{131} is in marked contrast to its slow disappearance after inoculation of iodinated or chromated serum proteins from a heterologous species (8).

The apparent heavy distribution of labelled endotoxin in the liver, buffy coat, lung, and spleen may be related to the ability of their phagocytic cells to remove endotoxin from the circulation. If the endotoxin in those tissues should exert no action locally or systemically, its removal would represent a protective function comparable to that exercised by the same organs when pathogenic bacteria are injected into the circulation. This protective removal of endotoxin might spare such targets as the brain. According to the important evidence of Penner and Klein (9), the brain mediates various systemic effects of endotoxin including hyperglycemia, hemoconcentration, intestinal edema, and even leukopenia. Our failure to detect radioactivity in the brains of rabbits receiving lethal doses of labelled endotoxin would suggest that the amount of endotoxin acting in the brain is too minute to detect, or that an agent which stimulates the brain might be elaborated after localization of endotoxin elsewhere.

It is possible that cerain manifestations may result from injury to those cells in which most of the endotoxin seemed to localize. For example, the apparent localization of chromium labelled endotoxin in the buffy coat, which contains leukocytes and platelets, may be related to the well known changes in leukocyte counts which take place whenever endotoxin is injected. It has also been demonstrated by Berthrong and Cluff (10) that centrifuged neutrophils lose their ability to migrate, and by Stetson (11) that leukocytes and platelets form thrombi in small vessels when rabbits are given endotoxin intravenously. Stetson has suggested that the adhesiveness of leukocytes increases after the injection of endotoxin.

These observations have suggested new lines of investigation which are now in progress: 1) To determine whether the cells which appear to remove endotoxin release substances upon injury which lead to the manifestations characteristic of lethal doses of endotoxin. Among the substances believed to be present in leukocytes are a pyrogen (12), and histamine (13); in hepatic cells, there is said to be both histamine and ferritin, a substance believed capable of inducing irreversible shock (14, 15); 2) To determine whether the development of tolerance depends on a greater ability of leukocytes and tissue macrophages to remove endotoxin from the circulation and thus

protect other targets; and on a greater capacity for the leukocytes themselves to resist its harmful effects. Evidence has already been provided by Cluff (16) that leukocytes can develop such resistance. He observed that centrifuged neutrophils of tolerant animals do not lose their ability to migrate after injection of endotoxin.

SUMMARY

On the basis of distribution of radioactivity, it appears that labelled endotoxin, after intravenous injection of lethal doses, was present in the plasma, but not erythrocytes, and then rapidly passed mainly to buffy coat and to liver. It accumulated in the buffy coat in high concentration during the period when leukopenia develops. In addition to leukopenia, the period of rapid removal from plasma was accompanied by fever, and sometimes by hypotension and diarrhea. The blood volume, as determined with Cr^{51}-labelled red cells, exhibited a variable response.

REFERENCES

1. Braude, A. I., Carey, F. J., Sutherland, D., and Zalesky, M., Studies with radioactive endotoxin. I. The use of Cr^{51} to label endotoxin of *Escherichia coli*. J. Clin. Invest., 1955, **34**, 850.
2. Grant, R. T., and Rothschild, P., A device for estimating blood-pressure in the rabbit. J. Physiol., 1934, **81**, 265.
3. Carey, F. J., Zalesky, M., and Braude, A. I., Studies with radioactive endotoxin. III. Distribution of radioactivity and associated physiologic changes in rabbits injected with lethal and sublethal doses of *E. coli* endotoxin labelled with radioactive chromic chloride. In preparation.
4. Carey, F. J., Zalesky, M., and Braude, A. I., Studies with radioactive endotoxin. IV. Effect of tolerance to *E. coli* endotoxin on the physiologic effects and distribution of radioactivity after injection of Cr^{51}-labelled endotoxin. In preparation.
5. Gitlin, D., Use of ultraviolet absorption spectroscopy in the quantitative precipitin reaction. J. Immunol., 1949, **62**, 437.
6. Seligman, A. M., Shear, M. J., Leiter, J., and Sweet, B., Chemical alteration of polysaccharide from *Serratia marcescens*. I. Tumor-necrotizing polysaccharide tagged with radioactive iodine. J. Nat. Cancer Inst., 1948, **9**, 13.
7. Barnes, F. W., Lupfer, H., and Henry, S. S., The biochemical target of Flexner dysentery somatic antigen. Yale J. Biol. & Med., 1952, **24**, 384.
8. Sterling, K., The turnover rate of serum albumin in man as measured by I^{131}-tagged albumin. J. Clin. Invest., 1951, **30**, 1228.
9. Penner, A., and Klein, S. H., The pathogenesis of experimental dysentery intoxication. Production of the lesions by cerebral circulation of the toxin. J. Exper. Med., 1952, **96**, 59.
10. Berthrong, M., and Cluff, L., Studies of the effect of bacterial endotoxins on rabbit leukocytes. I. Effect of intravenous injection of the substances with and without induction of the local Shwartzman reaction. J. Exper. Med., 1953, **98**, 331.
11. Stetson, C. A., Studies on the mechanism of the Shwartzman phenomenon. J. Exper. Med., 1951, **93**, 489.
12. Bennett, I. L., and Beeson, P. B., Studies on the pathogenesis of fever: II. Characterization of fever-producing substances from polymorphonuclear leukocytes and from the fluid of sterile exudates. J. Exper. Med., 1953, **98**, 493.
13. Chase, M. W., The allergic state *in* Bacterial and Mycotic Infections of Man, edited by Dubos, R. J., Philadelphia, J. B. Lippincott, 1948, p. 110–153.
14. Copenhaver, J. H., Jr., Nagler, M. E., and Goth, A., The intracellular distribution of histamine. J. Pharmacol. & Exper. Therap., 1953, **109**, 401.
15. Zweifach, B. W., Functional deterioration of terminal vascular bed in irreversible hemorrhagic shock. Ann. New York Acad. Sc., 1952, **55**, 370.
16. Cluff, L. E., Studies of the effect of bacterial endotoxins on rabbit leukocytes. II. Development of acquired resistance. J. Exper. Med., 1953, **98**, 349.

CARDIOVASCULAR AND RENAL RESPONSES TO THE COMBINATION OF HEXAMETHONIUM AND 1-HYDRAZINOPHTHALAZINE (APRESOLINE®) IN HYPERTENSIVE SUBJECTS [1]

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1-Hydrazinophthalazine has been demonstrated to cause a fall in peripheral artery pressure, to possess slight adrenergic blocking properties, to increase renal plasma flow (1–8), and to cause a proportional increase in cardiac output (8). It has been stated that the cardio-stimulating effects of 1-hydrazinophthalazine are mediated *via* the autonomic nervous system, since they can be blocked by sympathectomy or ganglionic blockade (9, 10).

These cardiac stimulating qualities of 1-hydrazinophthalazine may account for many of the undesirable side effects reported. Angina (11, 12), electrocardiographic changes (9), and myocardial infarction (13), have been reported during clinical therapy of hypertension with this agent. Though such evidence is at best only circumstantial, the use of 1-hydrazinophthalazine alone would seem to be of somewhat questionable value, particularly in the many patients suffering from hypertension complicated by coronary artery disease.

However, if the above side effects could be prevented by the prior administration of a ganglionic blocking agent, the combined use of 1-hydrazinophthalazine and hexamethonium, first proposed by Schroeder (14), would represent a satisfactory combination: the hypotensive qualities of 1-hydrazinophthalazine would be enhanced, while the cardiac stimulating effects would be blocked.

The purpose of the present study was twofold: (a) to evaluate the cardio-vascular and renal adjustments to 1-hydrazinophthalazine in a group of hypertensive patients; (b) to retest these adjustments when a ganglionic blocking agent, hexamethonium,[2] was administered just prior to the 1-hydrazinophthalazine.

MATERIAL AND METHODS

A total of eighteen patients with hypertension was studied. Three of these were thought to have hypertension secondary to renal disease, as determined by history and clinical evaluation. The remainder had "essential" hypertension.

A. *Experiments utilizing 1-hydrazinophthalazine alone*

Ten of the patients underwent a total of fifteen experimental studies with 1-hydrazinophthalazine alone. All were given the compound in rapid single intravenous doses of 0.20 to 0.50 mgm. per Kg. body weight. Repeated basal determinations of blood pressure and pulse were made before the 1-hydrazinophthalazine injection, and at intervals of 2 to 5 minutes thereafter for one to two hours.

1. *Renal clearance determination.* Renal plasma flow (C_{PAH}), and glomerular filtration rate (C_{IN}), were measured simultaneously in eight hypertensive patients by Smith's clearance techniques (15), but the data were not corrected for extraction values. The recorded data before administration of the drug represent the average of at least three successive urine collection periods of 12 to 20 minutes each. Following the administration of the drug, at least four similar successive urine collections were made.

2. *Hemodynamic studies.* Cardiac catheterization was performed in seven hypertensive patients after 1-hydrazinophthalazine. Five of these patients had renal clearance values determined just prior to the hemodynamic studies. Cardiac output was measured by the determination of oxygen consumption and A-V oxygen difference. Pulmonary and systemic arterial pressures were recorded by means of capacitance electromanometers or strain gauges.

B. *Experiments utilizing hexamethonium and 1-hydrazinophthalazine*

Ten of the patients underwent a total of seventeen experimental studies involving both these drugs.

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[2] Hexamethonium (Esomid®) supplied by Ciba Pharmaceutical Products, Inc.

1. *Renal clearance determination.* Using the same techniques, renal plasma flow and glomerular filtration rate were measured in nine patients before and after intravenous administration of 10 to 12.5 mgm. of hexamethonium ion, and again after the additional intravenous administration of 0.21 to 0.40 mgm. per Kg. of 1-hydrazinophthalazine. The latter drug was given within 45 to 60 minutes of the hexamethonium in each case. The clearance values represent the average of at least three 12 to 20-minute successive urine collections.

In one patient, T. G., the renal clearance determinations were measured simultaneously with the cardiovascular hemodynamic responses to the two drugs.

Only two of these nine patients had been studied before with 1-hydrazinophthalazine alone. Previous studies from this laboratory (8), however, would tend to indicate a fairly consistent renal response to this drug in hypertensive patients. In the previous series five of eight patients had responded with a significant rise in renal plasma flow following the 1-hydrazinophthalazine administration. (The other three patients had developed circulatory collapse.)

2. *Hemodynamic studies.* Using the techniques mentioned above, cardiac output and vascular pressures were obtained in patients before and after the administration of both hexamethonium and 1-hydrazinophthalazine. At least three determinations of cardiac output were made before and after the administration of each drug and were done at 10 to 15-minute intervals after stabilization of blood pressure readings. The 1-hydrazinophthalazine was given within 30 to 45 minutes after the hexamethonium in each case.

It should be emphasized that the effects of the two hypotensive agents were additive and alarming degrees of hypotension were encountered even in the recumbent position when 0.5 mgm. per Kg. of 1-hydrazinophthalazine was administered following the hexamethonium. In fact, five of the ten patients studied with the two drugs developed circulatory collapse and shock (E. C., O. W., A. L. P., V. G., I. B.). Each received only a moderate dose of 1-hydrazinophthalazine. It was, therefore, not prudent to insist on a full dose of 1-hydrazinophthalazine. It was thought, however, that definite increase in renal blood flow and in cardiac output had been satisfactorily demonstrated even with suboptimal doses of 1-hydrazinophthalazine (8), and for these reasons, the results of the present study were considered to be interpretable.

RESULTS

A. *Experiments using 1-hydrazinophthalazine alone*

1. *Renal clearances.* Average values for renal plasma flow, glomerular filtration rate, and filtration fraction in eight patients before and after the 1-hydrazinophthalazine administration are recorded in Table I together with the maximal changes in pulse rate and blood pressure.

TABLE I

Changes in B.P., pulse rate, C_{PAH}, C_{IN}, and F.F. in eight hypertensive subjects following intravenous administration of 1-hydrazinophthalazine

Pt.	Age	Sex	Dx	Dosage Mgm/Kilo	Dosage Mgm	B.P.	Pulse	C_{PAH}	% Chge.	C_{IN}	% Chge	F.F.	Comments
C.E.	57	M	E.H.	.40	24	170/110	76	552		98		.18	
						120/80	96	712	+29	85	-13	.12	
S.W.	54	M	E.H.	.35	22	174/102	72	430		93		.21	
						140/80	72	562	+30	74	-21	.15	
G.S.	48	M	E.H.	.40	25	190/130	72	369		88		.25	
						150/100	96	454	+23	89		.20	
P.W.*	39	M	E.H.	.20	15	140/80	76	541		125		.23	*This patient had been on oral Apresoline for several months with good result (See Discussion)
						110/64	84	458*	-16	106	-15	.23	
F.B.	40	M	R.H.	.20	14	250/130	72	220		58		.26	
						190/110	80	182	-17	55		.30	
E.C.	62	F	E.H.	.35	22	210/140	96	301		82		.27	**Marked Shock like picture.
						**66/60	64	62	-80	11	-87	.18	
E.K.	44	M	E.H.	.40	24	230/150	80	74		16		.21	
						140/70	96	36	-52	8	-50	.22	
G.K.	46	M	R.H.	.21	15	170/100	80	23		13		.59	
						170/90	90	22		22	+63	.41	

LEGEND
E.H. = Essential Hypertension
R.H. = Renal Hypertension

Values for C_{PAH} (renal plasma flow) expressed in c.c./Min/1.73 M^2 B.S.A.
Values for C_{IN} (glomerular filtration rate) expressed in c.c./Min/1.73 M^2 B.S.A.

The uppermost figure in each pair of figures represents the control value. The other figure corresponds to the maximal change following the drug.

TABLE II

Changes in cardiovascular hemodynamics in seven hypertensive subjects following intravenous administration of 1-hydrazinophthalazine

PT	AGE	SEX	DOSAGE MGM/KILO	MGM	C.O.	C.I.	% CHGE	PULSE	STR. VOL.	S.A.P.	P.A.P.
S.W.	54	M	.50	31	5.0	2.9		60	82	190/110	25/8
					10.9	6.5	+119	84	129	155/95	36/16
G.S.	48	M	.50	35	4.4	2.5		84	52	210/140	26/5
					10.0	5.9	+127	100	100	160/110	34/9
E.C.	62	F	.50	30	3.2	2.0		86	37	260/150	30/12
					4.0	2.5	+25	100	40	130/86	59/20
E.K.	44	M	.25	15	3.0	1.8		60	50	230/126	25/4
					5.2	3.1	+73	60	81	180/90	22/4
G.K.	46	M	.25	15	5.7	3.4		80	71	180/110	44/22
					8.2	4.9	+44	96	85	160/100	45/22
A.B.	34	M	.35	18	4.0	4.0		76	52	215/140	30/16
					7.3	7.3	+82	90	81	168/98	30/15
M.C.	49	F	.50	30	6.0	4.4		76	79	180/100	26/14
					7.1	5.2	+18	54	131	80/46	30/12

LEGEND

C.O. = Cardiac output in Litres/Min.
C.I. = Cardiac output/M² B.S.A.
STR. VOL. = Stroke volume
S.A.P. = Systemic arterial pressure
P.A.P. = Pulmonary arterial pressure

The uppermost figure in each pair of figures represents the control value. The other figure corresponds to the maximal change following the drug.

Four patients (G. S., C. E., S. W., P. W.) had normal or near normal resting values for renal plasma flow and glomerular filtration rate. Of these, three (G. S., C. E., S. W.) experienced an appreciable increase in renal plasma flow following intravenous injection of 1-hydrazinophthalazine. In the patient, P. W., renal plasma flow was observed to fall slightly after the drug had been administered. Either no change, or slight decline in glomerular filtration rate was noted in these four patients. In consequence, a decrease in the filtration fraction occurred in the three subjects who manifested a rise in renal plasma flow. In the other four patients (E. K., E. C., G. K., F. B.) control values for renal plasma flow and glomerular filtration rate were well below normal and none responded with an increase in renal plasma flow following the administration of the drug. Two of these patients (E. K., G. K.) had such extreme impairment of renal function that without correction for changes in extraction, the renal clearance values are recognizably inaccurate. However, these patients were purposely studied to determine whether or not 1-hydrazinophthalazine might have the reported effect in the presence of severe renal impairment. One might expect such patients to be the ones most likely to benefit from an increased renal blood flow. One patient, E. C., had moderate reduction of renal plasma flow before administration of the drug, but an acute hypotensive episode followed the injection of the 1-hydrazinophthalazine. In this subject values for renal plasma flow suggested virtual cessation of flow during this period.

2. *Hemodynamic studies.* Table II presents the hemodynamic changes observed on seven hypertensive patients. The average of three resting levels of cardiac output is compared with the maximal response within two hours after the 1-hydrazinophthalazine.

Five of the seven hypertensive patients (S. W., G. S., E. K., G. K., A. B.) experienced an obvious

rise in cardiac output following the drug injection. None of these patients had ever revealed clinical evidence of heart failure. All responded with a moderate decrease in arterial pressure with a concomitant rise in pulse rate. Only in one patient, G. K., was the mean pulmonary artery pressure elevated at rest and this level was not influenced by l-hydrazinophthalazine.

The other two patients (M. C., E. C.) did not demonstrate as striking a rise in cardiac output, although optimal doses of the agent were given. Both had recently exhibited evidence of heart failure. Another probable reason for the different responses in these two patients is that both of them developed hypotension with symptoms of shock. In E. C., who developed this shock-like state following the injection of .5 mgm. per Kg. of l-hydrazinophthalazine, a marked rise in mean pulmonary artery pressure occurred, suggesting either an episode of acute left ventricular failure or pulmonary venous spasm, or both. She recovered promptly and without treatment except oxygen inhalation. This same subject developed an identical state of collapse when given a

TABLE III

Changes in renal plasma flow (C_{PAH}), glomerular filtration rate (C_{IN}), and filtration fraction (F.F.) following single intravenous injections of Hexamethonium and l-hydrazinophthalazine (at 45-60 minute intervals).

PT.	AGE	SEX	DX	DOSAGE MGM/KILO HEXAM.	DOSAGE MGM/KILO HYDRAL	C_{PAH}	% CHGE	C_{IN}	% CHGE	F.F.	S.A.P.	PULSE
G.S.	48	M	E.H.			332		85		.17	190/130	68
				12.5		280	-21	69	-16	.20	155/110	68
					.25	415	+25	75	-12	.18	140/100	76
E.C.	62	F	E.H.			294		81		.27	220/108	66
				10		239	-19	72	-11	.30	180/100	56
					.28	298	NIL	69	-15	.23	150/80	80
A.P.	49	M	E.H.			534		121		.23	170/110	80
				12.5		444	-18	88	-27	.20	170/100	92
					.21	926	+73	124	NIL	.14	140/80	88
C.R.	46	M	E.H.			516		157		.30	170/110	80
				12.5		350	-32	84	-47	.24	140/100	96
					.25	716	+39	148	NIL	.20	134/94	96
M.C.	49	F	E.H.			300		52		.17	200/100	60
				10		238	-21	49	NIL	.20	180/80	76
					.25	210	-30	42	-17	.20	154/74	88
O.W.	65	F	E.H.			451		113		.25	210/110	72
				12.5		380	-16	80	-30	.21	160/100	80
					.25	282*	-37	52*	-54	.18	96/60	80
A.L.P.	61	M	E.H.			299		67		.22	180/100	64
				12.5		303	NIL	60	NIL	.20	170/100	64
					.27	267	-11	40	-40	.15	120/70	68
T.G.	46	M	E.H.			360		100		.29	190/130	72
				12.5		490	+38	145	+45	.29	175/115	76
					.40	425	+18	94	- 6	.22	150/100	80
I.B.	56	F	E.H.			306		85		.28	180/110	68
				12.5		234	-24	54	-36	.23	170/90	76
					.30	290	- 5	53	-36	.18	90/70	80

LEGEND

E.H. - Essential hypertension
HEXAM - Hexamethonium
HYDRAL - l-Hydrazinophthalazine
S.A.P. - Systemic arterial pressure

All Values for C_{PAH} and C_{IN} Expressed as cc/Min/1.73 Square Meters Body Surface Area. (The uppermost figure of each group of three represents the control value. The second figure represents the change after hexamethonium and the third figure, the change following l-hydrazinophthalazine.)

* Shock-like State Present.

TABLE IV

Changes in cardiovascular hemodynamics following single intravenous injections of Hexamethonium and 1-Hydrazinophthalazine in six hypertensive subjects (Injections 30-45 minutes apart).

PT.	AGE	DOSAGE HEXAM. MGM	DOSAGE HYDRAZ. MGM/KG	C.O.	C.I.	% CHGE	PULSE	STR. VOL.	S.A.P.	P.A.P.
G.S.*	48			5.3	3.0		64	83	170/120	25/5
		12.5		4.8	2.7		76	63	148/108	27/7
			.40	4.6	2.6		72	64	150/90	29/9
G.S.*	48			4.1	2.3		60	68	180/125	28/18
		12.5		3.8	2.2		84	45	170/107	22/5
			.50	6.1	3.5	+ 48	100	61	125/70	26/17
E.G.	62			2.1	1.3		66	32	190/110	35/14
		10		2.1	1.3		60	35	160/96	33/17
			.30	2.5	1.5		66	38	- 140/90	34/14
A.P.**	49			7.9	4.1		86	92	155/100	30/12
		10		7.1	3.7		92	77	120/80	18/10
			.22	7.7	4.0		96	80	120/70	21/11
A.P.**	49			6.8	3.7		90	75	185/120	15/5
		10		6.0	3.4		96	62	150/85	18/6
			.40	5.6	2.9		76	74	80/50	25/15
A.L.P.	62			4.1	2.2		76	54	150/100	25/7
		10		4.6	2.4		76	61	145/90	27/8
			.20	4.5	2.4		68	66	110/70	20/7
T.O.	46			5.8	2.7		64	90	180/120	34/15
		12.5		5.2	2.4		90	58	165/110	25/10
			.40	8.2	3.8	+ 40	76	100	125/70	25/10
V.O.	47			6.3	2.95		80	79	185/100	40/15
		12.5		6.2	2.9		84	74	140/80	28/10
			.40	***			66		80/60	

* - Same patient
** - Same Patient
*** - Severe shock. Procedure discontinued

LEGEND

HEXAM - Hexamethonium ion
HYDRAZ - 1-Hydrazinophthalazine
C.O. - Cardiac output in litres/min.
C.I. - Cardiac index in litres/min/M^2 body surface area
STR. VOL. - Stroke volume
S.A.P. - Systemic arterial pressure
P.A.P. - Pulmonary arterial pressure

The uppermost figure of each group of three represents the control value. The second figure represents the change after hexamethonium and the third figure, the change following 1-hydrazinophthalazine.

slightly smaller dose of the drug during evaluation of her renal function.

B. *Experiments using 1-hydrazinophthalazine and hexamethonium in combination*

Approximately 10 to 12.5 mgm. of hexamethonium were required in each case to produce autonomic blockade with a moderate reduction of the supine blood pressure. With this fall in blood pressure, little or no reflex rise in pulse rate was observed. The rise in pulse rate usually associated with 1-hydrazinophthalazine effect was absent when the hexamethonium had been administered first. This, we assumed, indicated at least some degree of blockade of sympathetic ganglia.

1. *Renal clearances.* Table III presents the changes in glomerular filtration rate and renal plasma flow (average of at least three samples), before hexamethonium, within 45 to 60 minutes

after hexamethonium and then within 60 to 75 minutes after 1-hydrazinophthalazine. Most of these nine patients experienced a slight fall in renal plasma flow and glomerular filtration rate following hexamethonium administration. These changes occurred simultaneously with a fall in blood pressure and have been reported previously (16–18). Following the 1-hydrazinophthalazine injection, only three of the patients (G. S., A. P., C. R.), experienced a significant rise in renal plasma flow. In these patients the control renal plasma flow levels were normal or nearly normal. In one patient (O. W.) the resting renal plasma flow was normal. However, on administration of the 1-hydrazinophthalazine, this patient became severely hypotensive and, like E. C., developed a shock like picture with further reduction of renal clearance values. No tachycardia was noted during the hypotensive period in any of these patients.

The remainder of the patients (M. C., E. C., A. P., T. G., I. B.) had reduced renal plasma flow prior to the drug injections. In one of these patients (E. C.) renal plasma flow fell slightly after the 1-hydrazinophthalazine and it did not change significantly in the remaining subjects.

It would seem, therefore, that patients capable of increasing renal plasma flow may do so and that this effect following 1-hydrazinophthalazine may not be blocked by hexamethonium in the range of dosage given. The blood pressure changes in the patients studied after the one drug and in those studied after the two drugs were comparable.

2. *Hemodynamic studies.* Table IV represents the cardiovascular adjustments observed in eight experiments on six hypertensive patients who were given both hexamethonium and 1-hydrazinophthalazine.

All patients experienced a fall in arterial pressure with little change in pulse rate. Two of the patients (A. L. P., V. G.) developed alarming hypotension lasting 3 to 4 hours after the 1-hydrazinophthalazine was given within 30 to 45 minutes of the hexamethonium. The expected rise in cardiac output following administration of 0.2 to 0.5 mgm. per Kg. of 1-hydrazinophthalazine was blocked, although incompletely in two patients (G. S. and T. G.) who received maximal doses of this drug. No changes in pulmonary artery pressure occurred. Two patients (G. S. and

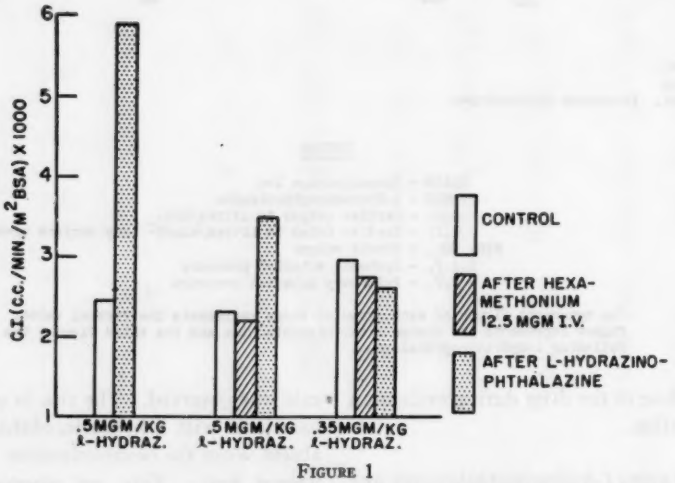

FIGURE 1

This 48-year-old male (G. S.) received a .5 mgm. per Kg. of 1-hydrazinophthalazine, i.v., which resulted in a striking increase in cardiac output. This effect could be partially blocked by hexamethonium. No increase in cardiac output was noted when the dose of 1-hydrazinophthalazine was slightly reduced after hexamethonium had been given. Injections of as little as 0.20 mgm. per Kg. of 1-hydrazinophthalazine always resulted in an appreciable elevation of cardiac output, (8), when not preceded by the administration of an autonomic blocking agent.

A. P.), were studied twice with different doses of the 1-hydrazinophthalazine each time. This was to demonstrate that within the dose range used the cardio-stimulating properties of 1-hydrazinophthalazine are blocked nearly completely by the hexamethonium.

Figure 1 records these changes in one hypertensive patient (G. S.) studied on three different occasions, first with 1-hydrazinophthalazine alone, then when hexamethonium was given prior to .5 mgm. per Kg. of 1-hydrazinophthalazine, and on a subsequent occasion when .35 mgm. per Kg. of 1-hydrazinophthalazine was administered after the hexamethonium.

DISCUSSION

1-Hydrazinophthalazine (Apresoline®) has been shown by several observers to increase renal plasma flow and cardiac output while reducing systemic arterial pressure (1–7). It has been suggested that these effects may also occur with oral administration of the drug (9).

The mechanism whereby these changes are produced is not clearly understood. It was thought that the increased renal plasma flow represented but another manifestation of the increased cardiac output (8). However, when the rise in cardiac output is partially or completely prevented by prior administration of a ganglionic blocking agent, the renal plasma flow may still increase in response to the 1-hydrazinophthalazine. This then may indicate that 1-hydrazinophthalazine has a selective effect on renal vessels which is independent of the level of cardiac output.

There is some evidence suggesting that only patients with relatively normal renal function are capable of responding to 1-hydrazinophthalazine by an increase in renal plasma flow. The patients with moderate or severe decrease in renal function did not experience a rise in estimated renal plasma flow following this drug. One might surmise that these subjects were incapable of responding to the drug by an increase in renal plasma flow because of irreversible vascular pathology.

While an increased renal plasma flow was associated in each case with a blood pressure decline, patients who developed severe hypotension invariably had a very marked fall in renal plasma flow regardless of the resting clearance level.

The lack of renal response to 1-hydrazinophthalazine given intravenously to a patient who had been receiving the drug orally for a long time (P. W., Table I) has been noted previously by Moyer (9). Here one might postulate that the oral medication had already resulted in a maximal renal-vascular response or that the patient had become insensitive to the drug.

In the patients receiving hexamethonium first, then 1-hydrazinophthalazine, the same theoretical mechanisms would seem to operate. Though the number of patients studied is too small to draw definite conclusions it is suggestive that only those patients with normal, or nearly normal, resting renal plasma flow were able to significantly increase their flow rates. 1-Hydrazinophthalazine, when administered parenterally, increases cardiac work by increasing cardiac output, even in the face of a decline in blood pressure. This, it appears, is true for normal subjects as well as for patients with systemic arterial hypertension. It has been noted with doses as low as 0.20 mgm. per Kg. We have observed that these properties of 1-hydrazinophthalazine can be blocked by prior administration of a small amount of hexamethonium given intravenously. The combination of the two drugs necessitated small doses of hexamethonium and in some instances doses of 1-hydrazinophthalazine which were smaller than those given to other subjects who had not been primed with hexamethonium. In all instances, however, dosages were given that remained within the effective level or which had produced effects in the same patient previously. The partial or complete blocking of the rise in cardiac output by a ganglionic blocking agent would indicate that these actions of 1-hydrazinophthalazine are mediated over the sympathetic nervous pathways.

This study may then offer a rational basis for such a therapeutic combination of drugs as has been proposed (11, 12, 14, 19, 20). The hypotensive effects are greater, the increase in renal plasma flow (when possible) still occurs, but the increase in cardiac output and cardiac work is partially or completely blocked. No information is available which would explain the observed increase in cardiac output following 1-hydrazinophthalazine and the blocking of this effect by hexamethonium. Following 1-hydrazinophthalazine widespread vasodilation of the arteriolar bed

seems to take place resulting in a decrease of total peripheral resistance in the face of increased total flow. Whatever mechanisms come into play, central, venomotor, or cardiac, they may perhaps be considered compensatory. Their interference by hexamethonium suggests that they are linked to the autonomic nervous system.

SUMMARY AND CONCLUSIONS

Eighteen hypertensive patients were studied in order to evaluate the renal and cardiovascular adjustments to 1-hydrazinophthalazine when given alone and after the administration of hexamethonium. The following observations were made:

1. 1-Hydrazinophthalazine seems to increase renal plasma flow only in patients with normal or nearly normal resting renal plasma flow.

2. The prior administration, parenterally, of a moderately hypotensive dose of hexamethonium, did not alter the ability of 1-hydrazinophthalazine to increase renal plasma flow in some patients.

3. 1-Hydrazinophthalazine caused an increase in cardiac output in all patients to whom it was given.

4. The prior administration of hexamethonium was capable of blocking the rise in cardiac output following the 1-hydrazinophthalazine.

REFERENCES

1. Reubi, F. C., Renal hyperemia induced in man by a new Phthalazine derivative. Proc. Soc. Exper. Biol. & Med., 1950, **73**, 102.
2. Grimson, K. S., Chittum, J. R., and Metcalf, B. H., Actions of 1-hydrazinophthalazine (C-5968), on vasomotor reflexes and hypertension in dogs and man. Federation Proc., 1950, **9**, 279.
3. Walker, H. A., Wilson, S., Atkins, E. C., Garrett, H. E., and Richardson, A. P., The effect of 1-hydrazinophthalazine (C-5968) and related compounds on the cardiovascular system of dogs. J. Pharmacol. & Exper. Therap., 1951, **101**, 368.
4. Moyer, J. H., Handley, C. A., and Huggins, R. A., Some pharmacodynamic effects of 1-hydrazinophthalazine (C-5968) with particular reference to renal function and cardiovascular response. J. Pharmacol. & Exper. Therap., 1951, **103**, 368.
5. Craver, B. N., and Yonkman, F. F., Some pharmacological properties of 1-hydrazinophthalazine, a hypotensive agent. Federation Proc., 1950, **9**, 265.
6. Walker, H. A., Wilson, S., and Richardson, A. P., Effect of 1-hydrazinophthalazine (C-5968) on the cardiovascular system of unanesthetized dogs. Federation Proc., 1950, **9**, 323.
7. Freis, E. D., and Finnerty, F. A., Jr., Supression of vasomotor reflexes in man following 1-hydrazinophthalazine (C-5968). Proc. Soc. Exper. Biol. & Med., 1950, **75**, 23.
8. Wilkinson, E. L., Backman, H., and Hecht, H. H., Cardiovascular and renal adjustments to a hypotensive agent. J. Clin. Invest., 1952, **31**, 872.
9. Moyer, J. H., Hydrallazine (1-Hydrazinophthalazine: CIBA BA-5968: Apresoline) hydrochloride, pharmacological observations and clinical results in the therapy of hypertension. Arch. Int. Med., 1953, **91**, 419.
10. Moyer, J. H., and Huggins, R. A., The effect of ganglionic blockage with hexamethonium on some of the pharmacodynamics of 1-hydrazinophthalazine. J. Pharmacol. & Exper. Therap., 1952, **106**, 407.
11. Schroeder, H. A., The effect of 1-hydrazinophthalazine in hypertension. Circulation, 1952, **5**, 28.
12. Morrow, J. D., Schroeder, H. A., and Perry, H. M., Jr., Studies on the control of hypertension by hyphex. II. Toxic reactions and side effects. Circulation, 1953, **8**, 829.
13. Grimson, K. S., Orgain, E. S., Rowe, C. R., and Sieber, H. A., Caution with regard to use of hexamethonium and "apresoline." J. A. M. A., 1952, **149**, 215.
14. Schroeder, H. A., Control of hypertension by hexamethonium and 1-hydrazinophthalazine, preliminary observations. Arch. Int. Med., 1952, **89**, 523.
15. Goldring, W., and Chasis, H., Hypertension and Hypertensive Disease. New York, The Commonwealth Fund, 1944, Appendix D.
16. Moyer, J. H., Huggins, R. A., Handley, C. A., and Mills, L. C., Effect of hexamethonium chloride on cardiovascular and renal hemodynamics and on electrolyte excretion. J. Pharmacol. & Exper. Therap., 1952, **106**, 157.
17. Mills, L. C., and Moyer, J. H., The acute effects of hexamethonium on renal hemodynamics in normotensive and hypertensive human subjects. Am. J. M. Sc., 1953, **226**, 1.
18. Moyer, J. H., and Mills, L. C., Hexamethonium—its effect on glomerular filtration rate, maximal tubular function, and renal excretion of electrolytes. J. Clin. Invest., 1953, **32**, 172.
19. Johnson, R. L., Freis, E. D., and Schnaper, H. W., Clinical evaluation of 1-hydrazinophthalazine (C-5968) in hypertension. Circulation, 1952, **5**, 833.
20. Schroeder, H. A., Morrow, J. D., and Perry, H. M., Jr., Studies on the control of hypertension by hyphex. I. Effect on blood pressure. Circulation, 1953, **8**, 672.

LOBAR ALVEOLAR GAS CONCENTRATION AFTER PNEUMONECTOMY[1]

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We have recently shown that end-expiratory lobar alveolar gas concentrations vary with body position (1). Erect man has higher oxygen and lower carbon dioxide concentrations and a higher R.Q. in the right upper lobe than in the right lower lobe. Supine man has no significant difference between these lobes in R.Q. but the oxygen concentration is lower and the carbon dioxide higher in the upper lobe than in the lower lobe.

Changes in lobar gas values in moving from the erect to the recumbent posture may be due to changes in pulmonary ventilation or perfusion. Pulmonary ventilation as a factor has been studied in subjects with pneumoperitoneum (2). With an increased tidal air-functional residual air ratio (3) there is supposedly more uniform ventilation (4, 5) but lobar differences in gas values are similar to those found in normal subjects. Thus a change in pulmonary ventilation with a change of body position does not appear to be the major factor.

To examine the role of pulmonary circulation, we have studied subjects following pneumonectomy. With total cardiac output passing through one lung, a more uniform pulmonary perfusion might be expected than in the normal subject.

METHOD

Five patients were selected who had a pneumonectomy three to twenty-four months before the experiment. All were women between the ages of 23 and 41 years. Four had undergone left pneumonectomy and one right. Four of the subjects had minimal disease or less in the remaining lung. The fifth (I. L.) is thought to have had no more than a minimal lesion in the remaining lung, but this cannot be positively ascertained. By X-ray there was no evidence of emphysematous bullae, but in all there was some scoliosis and over-distention of the remaining lung. All subjects had a thoracoplasty of 7 to 9 ribs on the pneumonectomy side. In three, a thoracoplasty had been unsuccessful in controlling the disease and a pneumonectomy followed. In two, the thoracoplasty followed the pneumonectomy to fill space. None had dyspnea on moderate effort; all felt in good health.

By the method previously described (1) small radiopaque plastic catheters (2 mm. o.d., 1 mm. i.d. 70 cm. long) were introduced into the upper lobe and lower lobe bronchus or a segmental division of these bronchi. Simultaneous end-expiratory gas samples were obtained from each lobe when the subject was erect and when recumbent. Gas samples were taken in pre-evacuated mercury sampling tubes and analyzed in a Scholander 0.5 cc. gas analyzer. At termination of the procedure a small bronchogram identified the position of the catheter tip.

Lung volume studies were completed on four subjects. The fifth was unavailable for this study. The Darling open circuit method (6) was used to determine the residual lung volume with the subject semi-recumbent.

RESULTS

Results in the remaining lung of five subjects with contralateral pneumonectomy are presented in Table I. Simultaneous end-expiratory oxygen and carbon dioxide values are shown for the upper and the lower lobe in each position. Respiratory exchange ratios were calculated for each lobe in both the supine and upright postures. All values are for the right side except in the one case whose remaining lung was on the left (I. L.). The paired samples method was used for all statistical analysis.

For the upright position nine paired samples on five subjects are shown. One of these was observed to be contaminated by inspiration. Upon elimination of this sample the oxygen concentration is higher in the upper lobe than in the lower lobe by a mean of 1.49 vol. per cent. This is statistically significant ($P = .01$). Carbon dioxide was lower in the upper lobe than in the lower lobe by 0.71 vol. per cent ($P < .01$). There is a mean difference between lobes in respiratory exchange ratio of 0.06, being higher in the upper lobe ($P < .01$).

For the supine position, Table I shows ten paired samples on five subjects. There was no

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TABLE I

Simultaneous upper and lower lobe alveolar air values in subjects with one lung (post-pneumonectomy) in the erect and supine positions

Subject	Barometer	Catheter	Upright				Horizontal					
			Volume %			Minutes upright before sample	Volume %			Minutes supine before sample	N_2 index	$\frac{RA}{TC} \times 100$
			O_2	CO_2	R.Q.		O_2	CO_2	R.Q.			
A. T.	756.0	RUL	14.27	5.24	0.75		12.19	5.84	0.62		2.6	53
		RLL	13.96	5.36	0.73	10	12.63	5.79	0.65	40	†	†
E. C.	760.0	RUL	18.60*	1.89	0.79		14.62	3.50	0.50			
		RLL	19.55*	1.09	0.77	5	14.84	5.12	0.81	20		
		RUL	15.74	4.26	0.79		†					
		RLL	15.23	4.50	0.75	10						
F. I.	757.1	RUL	16.95	2.90	0.69		16.48	4.01	0.89		2.2	52
		RLL	14.82	4.25	0.65	11	17.46	3.83	1.15	8		
		RUL	18.49	2.12	0.85		14.18	3.93	0.53			
		RLL	17.11	3.10	0.78	13	12.29	3.86	0.39	10		
I. L.	740.8	LUL	14.69	5.28	0.82		11.93	3.33	0.32		2.4	55
		LLL	12.96	5.87	0.69	5	14.67	4.92	0.75	7		
		LUL	16.78	3.39	0.79		12.81	5.56	0.64			
		LLL	13.00	5.86	0.69	10	13.80	5.51	0.73	11		
		LUL	†				12.87	5.79	0.67			
		LLL					13.01	5.78	0.68	45		
E. S.	752.9	RUL	16.15	4.15	0.85		15.23	4.90	0.84		2.3	46
		RLL	15.29	4.54	0.77	5	15.96	4.94	1.00	20		
		RUL	17.43*	2.91	0.80		13.76	5.16	0.67			
		RLL	14.94	4.78	0.76	9	14.49	5.17	0.77	27		
		RUL	14.12	5.13	0.71		16.04	4.50	0.91			
		RLL	12.90	5.64	0.65	15	16.27	4.80	1.05	5		

* Known contamination with inspired air.
† No samples taken.

observed contamination in this group but one sample is suspicious from its values. Using all values the oxygen concentration was lower in the upper lobe by a mean of 0.53 vol. per cent ($P < .05$). The carbon dioxide was lower in the upper lobe by a mean of 0.31 vol. per cent ($P > .10$). The R.Q. was lower in the upper lobe by 0.14 ($P = .02$).

Table I also shows the nitrogen index and residual air/total capacity ratios in four of the five subjects. All show high nitrogen indices for subjects with total ventilation in one lung and all show moderately elevated residual air total capacity ratios indicating pulmonary distention.

Table II shows a comparison of mean differences in gas concentration between upper and lower lobes for normals and subjects with but one lung. In the erect position, the subject with a pneumonectomy does not differ significantly from the normal in oxygen and carbon dioxide lobar differences. However, the mean difference between lobes in R.Q. is significantly smaller in the pneumonectomy case than in the normal subject ($P < .001$). When supine the mean lobar carbon dioxide difference in the pneumonectomy case is significantly smaller than in the normal subject, but the mean lobar oxygen difference is not significant. The lobar R.Q. differences for the supine pneumonectomy sub-

TABLE II

Comparison of mean differences between upper and lower lobe gas values in normal subjects and patients with but one lung *

	Upright			Supine		
	Volume %			Volume %		
	O_2	CO_2	R.Q.	O_2	CO_2	R.Q.
Normal	+2.14	−0.63	+0.18	−1.11	+0.42	−0.05
Subject with pneumonectomy	+1.49	−0.71	+0.06	−0.53	−0.31	−0.14

* Lower lobe values were subtracted from upper lobe values.

ject are not significantly different from values found in normal supine subjects.

The ventilation-perfusion equation (7) may be used to compare the two lobes where

$$\frac{V_A}{Q_c} = \frac{0.864\ R(Cc_{O_2} - C\bar{v}_{O_2})}{P_{A_{CO_2}}}$$

$(Cc_{O_2} - C\bar{v}_{O_2})$ is the arteriovenous oxygen difference in cc. per liter and should be sufficiently similar between lobes to regard it as a constant. The factor 0.864 is a constant and is the same for both lobes. For purposes of comparison between lobes, the variables are R and $P_{A_{CO_2}}$. The numerical values are of use only in comparing the lobes and are not included in the tables. In the erect pneumonectomy subject, the V_A/Q_c ratio is significantly higher in the upper lobe than in the lower lobe ($P < 0.01$). The same subject when supine shows a higher V_A/Q_c ratio in the lower lobe ($P = .05$).

Using the same equation, we have recalculated our original studies (1) in a similar manner. This has shown the V_A/Q_c ratio to be higher in the upper lobe than in the lower lobe ($P < .001$) when normal man is erect. When supine the lower lobe has the higher ventilation to perfusion ratio ($P < .02$). Comparison of normal and pneumonectomy subjects has shown no statistically significant difference in their mean lobar V_A/Q_c differences for the erect or supine position.

DISCUSSION

Lobar end-expiratory alveolar air studies in normal man (1) have shown the lower lobe to have a higher ventilation-perfusion ratio in the supine position. In contrast, erect man has a higher ventilation-perfusion ratio in the upper lobe. The difference between lobes may result from unequal ventilation or perfusion.

The object of the present study has been to determine the effect of altered pulmonary perfusion upon lobar alveolar air values. Subjects with but one lung might be expected to have more uniform pulmonary perfusion with total cardiac output flowing through one-half the normal vascular bed. More effective ventilation results from an increased tidal air functional residual air ratio in a single lung (5, 8). The present series of pneumonectomy subjects have both over-distention and high nitrogen indices with less effective ventilation than in a normal single lung. These subjects show alveolar gas R.Q. values to be higher in the upper lobe when erect. The lower lobe has higher R.Q. values when the subject is supine. The lobar difference in gas R.Q. when erect is significantly smaller in the pneumonectomy subject (0.06) than in the normal subject (0.17). Calculation of the V_A/Q_c ratio reveals the erect pneumonectomy subject to have a significantly higher value in the upper lobe than in the lower lobe. The supine subject has a higher ventilation to perfusion ratio in the lower lobe. These are not significantly different from values found in normal subjects, though total ventilation and blood flow are confined to one lung.

Considerable evidence has accumulated that total pulmonary ventilation is uneven (4, 6, 9, 10) in normal man. Little evidence exists that any division of the lung, such as a lobe, has more effective ventilation than other parts. Subjects with pneumoperitoneum (3) or post-pneumonectomy (5, 8) have increased tidal air/functional residual air ratios and more effective ventilation than normal man. They have similar lobar ventilation-perfusion differences. The part played by lobar ventilation in changing V_A/Q_c ratios with body position must await direct measurement, but the more important factor would seem to be perfusion.

Evidence that unequal perfusion exists is scant. Roelsen (9) believed unequal pulmonary perfusion exists in normal man, and others (11, 12) have assumed its existence in describing ventilation-perfusion relationships. In erect man, gravity could affect the low pulmonary arterial pressure to reduce blood flow through upper lobe. This would explain the high V_A/Q_c ratio in upper lobe when erect. Explanation of the higher lower lobe value when supine is not so apparent.

The relative prominence of either ventilation or perfusion in producing these results is not proven. Both factors may change in opposite directions from one position to another and present measurements are but net results.

Use of the V_A/Q_c equation in the manner above may be an approximation for lobes. In its use are assumptions that may apply more closely for the whole lung than for the individual lobe. Thus the lung is assumed to inspire gas

which it expired or of a homogeneous concentration from the dead space, which may not be true for the individual lobe. This problem is being further investigated.

CONCLUSIONS

Subjects with but one lung have higher ventilation-perfusion ratios in the upper lobe than in the lower lobe when erect. When supine the ventilation-perfusion ratio is higher in the lower lobe than in the upper. These values are not statistically different from those found in normals. The lobar gas R.Q. differences are significantly smaller in the pneumonectomy subject than in the normal subject in the erect position.

REFERENCES

1. Martin, C. J., Cline, F., Jr., and Marshall, H., Lobar alveolar gas concentrations; effect of body position. J. Clin. Invest., 1953, 32, 617.
2. Martin, C. J., Marshall, H., and Cline, F., Jr., Lobar alveolar gas concentrations; effect of reduced lung volumes. J. Applied Physiol., 1953, 6, 209.
3. Wright, G. W., Place, R., and Princi, F., The physiological effects of pneumoperitoneum upon the respiratory apparatus. Am. Rev. Tuberc., 1949, 60, 706.
4. Rauwerda, P. E., Unequal ventilation of different parts of the lung and the determination of cardiac output. Dissertation. Groningen Univ., Netherlands, 1946.
5. Cournand, A., Riley, R. L., Himmelstein, A., and Austrian, R., Pulmonary circulation and alveolar ventilation—perfusion relationships after pneumonectomy. J. Thoracic Surg., 1950, 19, 80.
6. Darling, R. C., Cournand, A., and Richards, D. W., Jr., Studies on the intrapulmonary mixing of gases. III. An open circuit method for measuring residual air. J. Clin. Invest., 1940, 19, 609.
7. Rahn, H., and Fenn, W. O., The oxygen-carbon dioxide diagram. WADC Technical Report, 1953, 255, 14.
8. Gaensler, E. A., and Strieder, J. W., Progressive changes in pulmonary function after pneumonectomy: The influence of thoracoplasty, pneumothorax, oleothorax and plastic sponge plombage on the side of pneumonectomy. J. Thoracic Surg., 1951, 22, 1.
9. Roelsen, E., The composition of the alveolar air investigated by fractional sampling. Comparative investigations in normal persons and patients with bronchial asthma and pulmonary emphysema. Acta. med. Scandinav., 1939, 98, 141.
10. Fowler, W. S., Lung function studies. III. Uneven pulmonary ventilation in normal subjects and in patients with pulmonary disease. J. Applied Physiol., 1949, 2, 283.
11. Rahn, H., A concept of mean alveolar air and the ventilation-blood flow relationships during pulmonary gas exchange. Am. J. Physiol., 1949, 158, 21.
12. Riley, R. L., and Cournand, A., 'Ideal' alveolar air and the analysis of ventilation-perfusion relationships in the lungs. J. Applied Physiol., 1949, 1, 825.

BLOOD VOLUME IN CONGESTIVE HEART FAILURE[1]

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Discussions of the mechanism of congestive heart failure usually involve a consideration of the blood volume (1, 2). The problem has engaged the attention of many investigators: some have found blood volume to be increased in congestive heart failure (3–7); others have found it to be normal (8, 9). This report is made to record the authors' observations in a study of 102 patients in heart failure.

In this investigation the answers to three questions were sought: 1. Is the blood volume of patients in congestive heart failure different from the blood volume of control patients without heart disease? 2. (a) Is there a change in blood volume as patients improve from heart failure to the compensated state? (b) If so, can this change be attributed solely to improvement in circulatory function or may it be due to bed rest alone? There are several reports that a reduction in blood volume occurs with inactivity or bed rest (10–12). 3. Is there a change in blood volume in patients who revert spontaneously from a state of compensation to heart failure? In the literature there are many answers to questions 1 and 2(a) but little information relative to questions 2(b) and 3. In our study particular attention has been devoted to these two questions.

METHODS

Blood volume studies were made on 102 patients in heart failure and on 107 control patients. The absolute criterion for the diagnosis of congestive heart failure was unequivocal elevation of jugular venous pressure as determined by clinical examination. No patient was included in this series in whom this finding was absent or questionable. These patients had, in addition, the other signs of heart failure: orthopnea, basal râles, cardiac and hepatic enlargement, and peripheral edema. A few lacked detectable edema but presented all the other manifestations; some of the patients with pulmonary heart disease did not have basal râles. All the patients were acutely ill and presented the clinical picture of advanced "right-sided" heart failure. The etiological types of heart disease and their frequency were: arteriosclerotic, 34; hypertensive and arteriosclerotic, 26; hypertensive, 14; rheumatic, 14; pulmonary, 11; congenital, 1; luetic, 1; amyloid, 1.

An initial blood volume determination was made usually within twelve hours of admission to hospital. In as many cases as possible it was repeated when all clinical signs of failure had disappeared.

Control patients were selected who did not have any known disease of heart, lungs or blood and who were not acutely ill. The commonest diagnoses in this group were diabetes, peptic ulcer, epilepsy and other neurological disorders, and convalescent pneumonia. In fifty-five of the control patients who remained largely confined to bed during their hospital stay, an initial blood volume determination was made shortly after admission and was repeated after a varying interval of time. These patients were not strictly immobilized; some had bathroom privileges. It was considered that their degree of activity did not differ markedly from that of patients recovering from heart failure. These patients were not controls in the strictest sense of that term because they did not have dietary salt restriction, digitalis and diuretics as did the patients in congestive heart failure. The repeat blood volume determinations on these subjects provided a group of paired observations to test the reproducibility of the method and to show whether significant changes in blood volume would result from ordinary bed rest.

In some of the cardiac patients the opportunity occurred to measure the blood volume at the time of readmission in heart failure, their blood volume having been determined on a prior occasion in the compensated state. An interval of several months often separated the two admissions. No control observations were made for this group.

Blood volume was determined by the P^{32} tagged red blood cell method. Eight or ten cubic centimeters of the subject's blood were incubated for forty-five to sixty minutes at 37° C. with 50 to 75 microcuries of P^{32} in a sterile rubber-capped tube. The plasma was removed after centrifugation and the cells washed twice with sterile isotonic saline solution. The cells were then resuspended in saline to approximately the original volume and, without delay, a 5.0 cc. aliquot was injected intravenously from a calibrated syringe. One venous blood sample was drawn from the opposite arm vein ten to fifteen minutes after injection, using dry powdered heparin as the anticoagulant. It was found, using this

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technique of preparation, that the amount of P^{32} in the supernatant saline of the final tagged-cell suspension comprised approximately 2 per cent of the injected dose by comparison against a standard uranium source. No correction was made for this extra-cellular P^{32} in the calculation. It was found also, as reported by others (6), that the radioactivity of venous blood measured at fifteen-minute intervals for a period of one hour after the intravenous injection did not vary significantly from the ten to fifteen-minute value even in patients with heart failure. In the routine procedure the single sample was therefore used, it being considered that mixing was complete at that time.

In the counting technique liquid samples were used. One cubic centimeter of the tagged-cell suspension ("standard") was first hemolyzed and then diluted to 200 cc. in 18 per cent dextrose, as described by Reid and Orr (13). The venous blood sample was untreated. These samples were placed in specially prepared pyrex glass beakers of identical internal diameter mounted horizontally below the end window of a Geiger tube. The depth of the fluid in the beakers was 12 mm. so that the principle of "infinite thickness" was employed (13). Five thousand to six thousand counts were recorded at a rate of counting twenty times background. Counting of the venous blood sample was interrupted at five-minute intervals for stirring to prevent settling of intact red cells away from the important upper layers of fluid. The hematocrit of venous blood was read from duplicate samples after centrifugation at 3,000 r.p.m. for forty minutes. No correction was applied for trapped plasma. The calculations were made as follows:

$$\text{Total Blood Volume cc.} = \frac{\text{counts per min. (standard)} \times 5.0 \text{ (vol. injected)} \times 200 \text{ (dil. factor)}}{\text{counts per min. (venous sample)}}$$

Red Cell Volume = Total Blood Volume × Hematocrit

Plasma Volume = Total Blood Volume − Red Cell Volume.

The technical accuracy of the method was tested *in vitro* on bottles of blood, care being taken to arrange that the amount of radioactivity in the samples was similar to that in the measurements on humans. In nine successive experiments the mean difference of the determined from actual volume was −0.06 per cent (Standard Deviation ±4.08 per cent). This test of accuracy does not include physiological errors. It is merely a test of the accuracy of the diluting and counting techniques.

Because this is a tagged red cell method, only the red cell volume will be directly measured. The values for total volume and plasma volume are dependent on the venous hematocrit. Because total body hematocrit is probably lower than venous hematocrit (14–16) values given here for total volume and plasma volume may be low. In this report conclusions are based on differences in volumes measured by identical methods. If the ratio of body to venous hematocrit is the same in control patients, patients in heart failure, and patients in the compensated state, the conclusions based on the differences observed are valid. The important observation (6) that the ratio of venous to body hematocrit may not be identical in these three groups of patients will be considered in the discussion.

RESULTS

Heart failure group versus control group

The average values of total, red cell, and plasma volume for the group of 102 patients in heart failure are compared with those of 107 control patients in Table I. Males in heart failure have an excess of 983 cc. of total volume over the controls; females an excess of 1,313 cc. Since the hematocrit values are very nearly identical in the two groups, the excess is shared by the red cell and plasma fractions in about the same proportion as in normal blood.

The values for total, red cell and plasma volumes, expressed in cc. per kilogram of body weight, of the 107 control patients and of the survivors in the heart failure group in whom it was possible to determine "dry" body weight in the compensated state are given in Table II. Males in heart failure had a mean total blood volume of 90.7 cc. per Kg.; male controls 69.8 cc. per Kg. Females in heart failure had a mean total blood volume of 88.2 cc. per Kg.; female controls 60.6 cc. per Kg. The mean differences were significant: males, $t = 7.9$; $P < .001$; females, $t = 6.5$; $P < .001$.

TABLE I

Comparison of blood volume: Heart failure vs. controls *

	Blood volume (expressed in cc.)		
	Controls	Heart failure	Excess: Failure over controls
Males	75 Cases	70 Cases	
Total B.V.	4,545	5,528	+983
Red cell V.	2,018	2,515	+497
Plasma V.	2,527	3,013	+486
Hematocrit	44.4%	45.5%	
Average age	49.5 yr.	64.5 yr.	
Females	32 Cases	32 Cases	
Total B.V.	3,555	4,868	+1,313
Red cell V.	1,477	2,041	+564
Plasma V.	2,078	2,827	+749
Hematocrit	41.6%	42.0%	
Average age	53.3 yr.	58.7 yr.	

* Average values.

TABLE II

Total, red cell and plasma volume of control patients and of cardiac patients in failure and free of failure expressed as cubic centimeters per kilogram of body weight *

	Control group			Failure group			
				In failure		Free of failure	
	Mean	S.D.†		Mean	S.D.	Mean	S.D.
Males 75 Cases			Males 46 Cases				
Total B.V.	69.8	±10.8		90.7	±18.3	80.4	±13.3
Red cell V.	30.9			40.4		38.8	
Plasma V.	38.9			50.3		41.6	
Hematocrit	44.2			44.6		48.3	
Females 32 Cases			Females 18 Cases				
Total B.V.	60.6	± 8.9		88.2	±21.1	70.5	±13.9
Red cell V.	25.1			35.9		31.7	
Plasma V.	35.5			52.3		38.8	
Hematocrit	41.6			40.7		45.0	

* Values for cardiac patients based on failure-free or "dry" weight.
† S.D. = Standard Deviation.

Frequency distribution diagrams of total blood volume in cubic centimeters per kilogram in these same groups are shown in Figures 1 and 2. Although patients in congestive heart failure had an average total blood volume significantly greater than patients without heart disease, there were some patients in heart failure whose total blood volume was within the range of normal.

Heart failure to compensation versus repeat determinations in controls

In the control group of 55 patients who remained at bed rest for an average period of 9.5 days there was no significant difference between the averages of the initial and repeat determinations. In the congestive heart failure group of 78

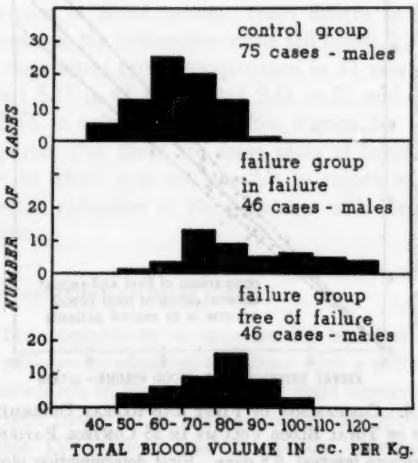

FIG. 1. FREQUENCY DISTRIBUTION DIAGRAMS OF TOTAL BLOOD VOLUME IN CUBIC CENTIMETERS PER KILOGRAM OF BODY WEIGHT IN TWO GROUPS OF MALE PATIENTS: 1) CONTROLS; 2) CARDIAC PATIENTS IN FAILURE AND FREE OF FAILURE

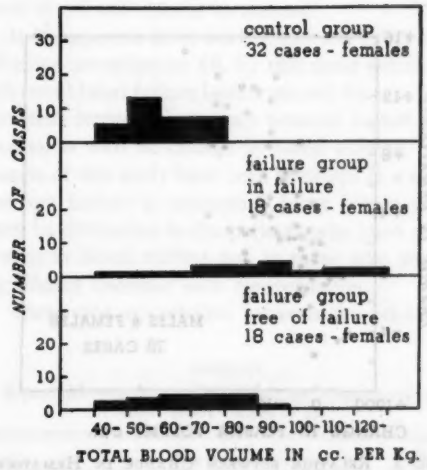

FIG. 2. FREQUENCY DISTRIBUTION DIAGRAMS OF TOTAL BLOOD VOLUME IN CUBIC CENTIMETERS PER KILOGRAM OF BODY WEIGHT IN TWO GROUPS OF FEMALE PATIENTS: 1) CONTROLS; 2) CARDIAC PATIENTS IN FAILURE AND FREE OF FAILURE

TABLE III

Repeat determinations of blood volume in heart failure and control cases *

	Volume (average values)		
	In failure	Free of failure	Loss or gain
Heart failure 78 Cases			
Total B.V.	5,598	4,888	−710
Red cell V.	2,463	2,340	−123
Plasma V.	3,135	2,548	−587
Hematocrit	44.1%	47.9%	
Controls 55 Cases	I	II	
Total B.V.	4,089	4,170	+81
Red cell V.	1,765	1,797	+32
Plasma V.	2,324	2,373	+49
Hematocrit	43.1%	42.9%	

* Blood volume expressed in cubic centimeters.

comparisons, where the average interval was 17.9 days between the initial determination in congestive failure and the repeat determination free of failure, there was a mean reduction of total blood volume of 710 cc. (See Table III.) This was due chiefly to a reduction in plasma volume with an accompanying rise in hematocrit from 44.1 per cent in failure to 47.9 per cent in the compensated state. The relation between rise in hematocrit and decrease in plasma volume in individual cases is shown in Figure 3.

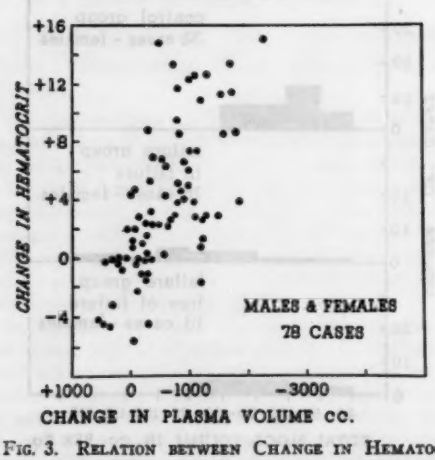

FIG. 3. RELATION BETWEEN CHANGE IN HEMATOCRIT AND CHANGE IN PLASMA VOLUME DURING RECOVERY FROM HEART FAILURE

On the ordinate scale change in hematocrit is plotted from the direct reading, *e.g.*, 40 per cent in failure to 50 per cent free of failure is plotted as +10.

The individual values from these two series of repeat determinations are plotted in the diagrams of Figures 4 and 5. For statistical treatment the individual differences in total volume were expressed as percentage of the initial value. For the control group, the mean difference was +2.8 per cent (S.D. ± 10.6 per cent); for the failure group, it was −11.3 per cent (S.D. ± 13.6 per cent). Application of the *t* test revealed that the difference between the means was highly significant ($t = 7.0$; $P < .001$).

These results, expressed in terms of cubic centimeters per kilogram of body weight (Table II), showed that males had a mean reduction of total blood volume from 90.7 cc. per Kg. in heart failure to 80.4 cc. per Kg. in the compensated state. Females showed a reduction from 88.2 cc. per Kg. in failure to 70.5 cc. per Kg. in the compensated state. These mean reductions were significant: (Males, $t = 3.35$; $P < .01$); (Females, $t = 2.53$; $P < .05$).

In this group of patients followed from congestive heart failure to compensation, there was a significant average reduction in total blood volume compared with a control group. This general statement can be given strong statistical sup-

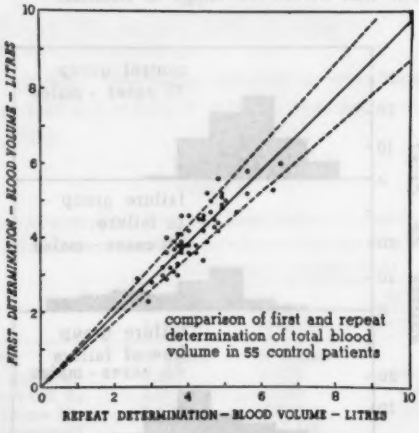

FIG. 4. COMPARISON OF FIRST AND REPEAT DETERMINATION OF TOTAL BLOOD VOLUME IN 55 CONTROL PATIENTS

Average interval, 9.5 days. First determination plotted as the ordinate; repeat determination as the abscissa. The solid line passes through the mean of the initial *versus* repeat determinations. The interrupted lines embrace ±1 standard deviation of the individual differences about that mean.

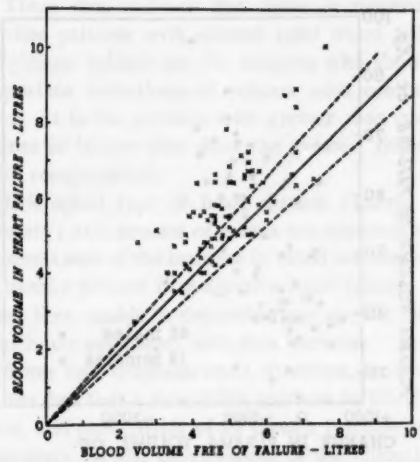

FIG. 5. COMPARISON OF BLOOD VOLUME IN FAILURE WITH BLOOD VOLUME FREE OF FAILURE IN 78 CASES

Average interval 17.9 days. Blood volume in failure plotted as the ordinate; blood volume free of failure as the abscissa. Solid and interrupted lines are those of the control group in Figure 4. Points which fall above the solid line indicate a decrease in blood volume with compensation.

port but it does not imply that in each individual case of heart failure there was a reduction of blood volume with compensation. Of the 78 comparisons of blood volume, heart failure to compensation, the failure-free value fell below 2 S.D.'s of the control group comparisons in 21 cases, below 1 S.D. in 42, within ± 1 S.D. in 25, and above 1 S.D. in only one case. (See Figure 5.) It is apparent that there are some cases of heart failure in which it is not possible to record an important reduction of blood volume with compensation.

Compensation to heart failure

The opportunity to compare the blood volume of patients re-admitted in failure with a prior determination in the compensated state occurred in sixteen cases. The average values are given in Table IV. There was an average increase in total blood volume of 510 cubic centimeters. Calculated on the basis of percentage increase in individual cases, the mean percentage increase was 11.9 per cent and was significant ($t = 2.32$; $P < .05$). This increase was due almost entirely to plasma; the red cell volume remained unchanged and the hematocrit fell from 49.8 per cent to 45.2 per cent with hemodilution. These changes are the reverse of those observed during recovery from heart failure where the plasma volume decreased and the hematocrit rose. Although there was a statistically significant mean increase, in eight cases the changes were slight, in eight there were large increases, and in none were there large decreases. There was no correlation between the size of the increase and the interval of time separating the two measurements.

DISCUSSION

The observations reported here indicate that in general the blood volume in congestive heart failure is greater than normal. This excess of blood is composed about equally of red cells and plasma. The plasma fraction decreases with compensation and increases again with congestive heart failure.

The red cell volume shows a small but statistically significant reduction with cardiac compensation. It is reasonable to assume that decrease in red cell mass would occur slowly, and that the average interval of eighteen days between the two determinations was not long enough to detect return of red cell volume to normal.

It is apparent from our results and from those of other investigators (8, 9) that some patients in advanced heart failure have a normal blood volume and that some patients may proceed to full compensation with no change in blood volume. The results of this study have been reviewed in a search for any factors in congestive heart failure which may be distinctive to the patients who have an increase in blood volume and to those who show a significant decrease with compensation.

There was no relation between the etiological

TABLE IV

Repeat determinations of blood volume: Compensation to heart failure—mean of 16 *

	Free of failure	In failure	Loss or gain
Total B.V.	4,969	5,479	+510
Red cell V.	2,475	2,480	+5
Plasma V.	2,494	2,999	+505
Hematocrit	49.8%	45.2%	

* Blood volume expressed in cubic centimeters.

type of heart disease and either initial level of blood volume or the decrease with compensation except in pulmonary heart disease where the red cell volume was high.

Degree of heart failure in the sense of "left-sided" or "right-sided" did not bear on the problem since all the cases were of advanced "right-sided" congestive failure with all evidences of systemic venous congestion where that was primarily of left ventricular, valvular or pulmonary origin.

It seemed reasonable to believe that grossly edematous patients with their obvious excess of total body fluid would always have initially high plasma volumes and would show significant reduction of plasma volume after removal of the edema fluid, and that the reverse would hold for patients with little edema. It was, in fact, part of the theory of Warren and Stead (2) that plasma volume and extra-cellular fluid volume increased and decreased concordantly. To study this question the loss in body weight during recovery from congestive heart failure has been considered an approximate measure of the amount of edema originally present. The correlation between this loss in weight and the value for plasma volume in failure, in cubic centimeters per kilogram, is shown in Figure 6. There is no significant correlation: $r = .084$; $t = .645$; $P > 0.5$. Some patients with very little edema have a high plasma volume; other patients with massive edema have a normal plasma volume. By similar comparison, no correlation existed between the original amount of edema and degree of reduction of plasma volume during recovery from heart failure.

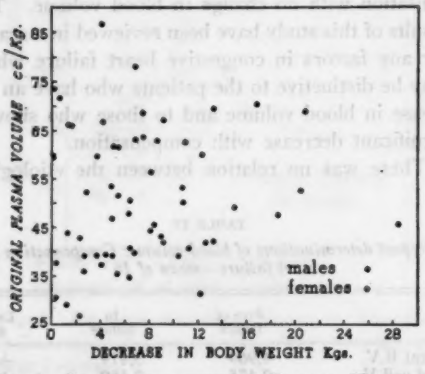

FIG. 6. CORRELATION BETWEEN PLASMA VOLUME IN HEART FAILURE IN CUBIC CENTIMETERS PER KILOGRAM AND DECREASE IN BODY WEIGHT DURING RECOVERY FROM HEART FAILURE

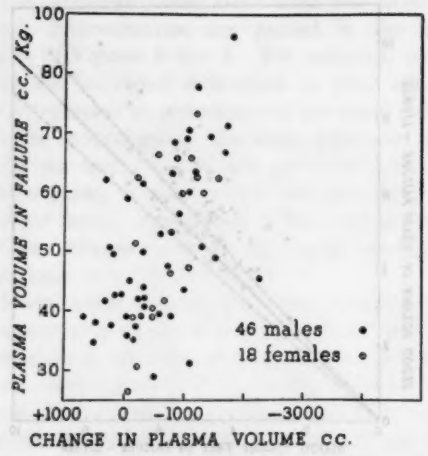

FIG. 7. CORRELATION BETWEEN PLASMA VOLUME IN HEART FAILURE, IN CUBIC CENTIMETERS PER KILOGRAM, AND ABSOLUTE CHANGE IN PLASMA VOLUME IN CUBIC CENTIMETERS DURING RECOVERY FROM HEART FAILURE

Because there were some decompensated cardiac patients with values for blood volume within the normal range and some who failed to show a reduction of blood volume with compensation, it seemed important to determine whether these two groups were composed for the most part of the same patients. For the group of patients whose total blood volume in heart failure was within the normal range (below 90 cc. per Kg.; see Figures 1 and 2) the mean reduction of blood volume with compensation was 6.1 per cent. For the patients whose total blood volume in heart failure was above the normal range (90 cc. per Kg. or greater), the mean reduction with compensation was 16.5 per cent.

The correlation between original plasma volume in congestive failure, in cubic centimeters per kilogram, and absolute reduction in plasma volume, in cubic centimeters, was tested. (See Figure 7.) This correlation was significant ($r = 0.54$; $t = 5.4$; $P < .001$). The correlation between original plasma volume in failure and per cent reduction of plasma volume with compensation was also significant: ($r = .45$; $t = 4.3$; $P < .001$).

These data indicate that those decompensated cardiac patients with normal total blood volume or plasma volume are the subjects who show unimportant reductions of volume with compensation. It is the patients with greater than normal values in failure who show the greatest reduction with compensation.

Etiological type of heart disease (except pulmonary) and amount of edema are apparently not determinants of the increase in blood volume which is usually present in congestive heart failure. We have been unable to determine any specific factors which are associated with this increase.

Some final emphasis must, therefore, be directed to the fact that a recordable increase in blood volume, and particularly of its plasma fraction, is not necessary for the development of the clinical picture of congestive heart failure even though it be the usual finding. "Congestion" may occur with a redistribution of blood to the venous side without an increase in blood volume.

Schreiber, Bauman, Yalow, and Berson (6) have reported that the mean ratio of body to venous hematocrit is .937 in control patients, .868 in patients with congestive heart failure, and .908 in patients free of failure. These differences in ratio imply that, where a tagged red cell method is used to determine total blood volume, comparisons between control patients, cardiac patients in congestive heart failure, and cardiac patients in the compensated state would be in error. The tendency would be to underestimate the differences: control *versus* heart failure; heart failure *versus* compensation.

We have reviewed our data with respect to the above observations. The net effect is to accentuate the mean differences already observed. That is, that blood volume is greater than normal in heart failure; that there is significant mean decrease with compensation; that there is significant mean increase on reversion to the state of failure. The correlations in Figures 6 and 7 remain virtually unchanged. However, there are still individual patients whose blood volume in heart failure is within the normal range; patients who proceed to compensation without significant change in blood volume; and patients who revert from compensation to heart failure without significant change.

SUMMARY

Blood volume studies by the P^{32} tagged red blood cell method have been made on 102 patients in congestive heart failure and on 107 control patients.

Patients in congestive heart failure usually have a larger blood volume than controls.

With compensation there is usually a decrease in blood volume, principally of its plasma fraction.

With the spontaneous reappearance of congestive failure, the blood volume usually increases again.

Etiological type of heart disease and amount of edema do not determine the blood volume changes in heart failure, except pulmonary heart disease where the red cell volume is high.

In some cases of congestive heart failure blood volume may be normal, may not decrease with compensation or increase with the reappearance of failure.

ACKNOWLEDGMENTS

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REFERENCES

1. Peters, J. P., The problem of cardiac edema. Am. J. Med., 1952, **12**, 66.
2. Warren, J. V., and Stead, E. A., Jr., Fluid dynamics in chronic congestive heart failure. An interpretation of the mechanisms producing the edema increased plasma volume and elevated venous pressure in certain patients with prolonged congestive failure. Arch. Int. Med., 1944, **73**, 138.
3. Gibson, J. G., 2nd, and Evans, W. A., Jr., Clinical studies of the blood volume. III. Changes in blood volume, venous pressure and blood velocity rate in chronic congestive heart failure. J. Clin. Invest., 1937, **16**, 851.
4. Meneely, G. R., and Kaltreider, N. L., A study of the volume of the blood in congestive heart failure. Relation to other measurements in fifteen patients. J. Clin. Invest., 1943, **22**, 521.
5. Seymour, W. B., Pritchard, W. H., Longley, L. P., and Hayman, J. M., Jr., Cardiac output, blood and interstitial fluid volumes, total circulating serum protein and kidney function during cardiac failure and after improvement. J. Clin. Invest., 1942, **21**, 229.

6. Schreiber, S. S., Bauman, A., Yalow, R. S., and Berson, S. A., Blood volume alterations in congestive heart failure. J. Clin. Invest., 1954, 33, 578.
7. Reilly, W. A., French, R. M., Lau, F. Y. K., Scott, K. G., and White, W. E., Whole blood volume determined by radiochromium-tagged red cells. Comparative studies on normals and congestive heart failure patients. Circulation, 1954, 9, 571.
8. Prentice, T. C., Berlin, N. I., Hyde, G. M., Parsons, R. J., Lawrence, J. H., and Port, S., Total red cell volume, plasma volume, and sodium space in congestive heart failure. J. Clin. Invest., 1951, 30, 1471.
9. Ross, J. F., Chodos, R. B., Baker, W. H., and Freis, E. D., The blood volume in congestive heart failure. Tr. A. Am. Physicians, 1952, 65, 75.
10. Taylor, H. L., Erickson, L., Henschel, A., and Keyes, A., The effect of bed rest on the blood volume of normal young men. Am. J. Physiol., 1945, 144, 227.
11. Deitrick, J. E., Whedon, G. D., and Shorr, E., Effects of immobilization upon various metabolic and physiological functions of normal men. Am. J. Med., 1948, 4, 3.
12. Sjöstrand, T., Volume and distribution of blood, and their significance in regulating the circulation. Physiol. Rev., 1953, 33, 202.
13. Reid, A. F., and Orr, M. K., A rapid method for determining blood volumes by the use of P^{32}-labelled red cells. J. Clin. Invest., 1950, 29, 313.
14. Smith, H. P., Arnold, H. R., and Whipple, G. H., Blood volume studies. VII. Comparative values of Welcker, carbon monoxide and dye methods for blood volume determinations. Accurate estimation of absolute blood volume. Am. J. Physiol., 1921, 56, 336.
15. Gibson, J. G., 2nd, Peacock, W. C., Seligman, A. M., and Sack, T., Circulating red cell volume measured simultaneously by the radioactive iron and dye methods. J. Clin. Invest., 1946, 25, 838.
16. Gray, S. J., and Frank, H., The simultaneous determination of red cell mass and plasma volume in man with radioactive sodium chromate and chromic chloride. J. Clin. Invest., 1953, 32, 1000.

A COMPARISON OF THE RELATIVE EFFECTIVENESS OF HYDROPENIA AND OF PITRESSIN® IN PRODUCING A CONCENTRATED URINE [1, 2]

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It is a common assumption that exogenous anti-diuretic hormone (Pitressin®) is equal to the endogenous anti-diuretic hormone (ADH) in its ability to stimulate the production of a concentrated urine. However, clinical studies of urine formation after the administration of Pitressin® or Pituitrin® leave some doubt as to the ability of the exogenous hormone to produce a maximally concentrated urine. Sodeman and Engelhardt (1) found that if urinary specific gravity was low at the time of Pituitrin® administration, the value attained was not so high as could be produced in the same subject by water deprivation. Similar observations were made by Little, Wallace, Whatley, and Anderson (2). Taylor, Peirce, and Page (3) gave Pituitrin® at frequent intervals for 12 hours and found the urine to be often more dilute and of larger volume than after a 12-hour period of water deprivation. In every case the amounts of anti-diuretic hormone administered were much greater than are assumed to be normally produced.

In the present study the ability of exogenous anti-diuretic hormone, administered as Pitressin®, to concentrate the urine has been reinvestigated in humans and in the dog. Because it is now recognized that the concentration of urine depends not only on the degree of hydropenia, but also on the prevailing rate of solute excretion (4–6), it seemed pertinent to investigate urinary concentration after Pitressin® administration during mild solute diuresis as well as under normal conditions of urine formation. To insure absence of circulating endogenous anti-diuretic hormone at the time Pitressin® was tested, the subjects were hydrated and having a water diuresis at the start of hormone infusion. In evaluating the results, the urinary concentrations after Pitressin®, expressed in milliosmols, have been compared with the urine osmolarity of hydropenic subjects, taking into account the fall in osmolarity normally occurring during solute diuresis.

It was found that once water diuresis was inhibited by the administration of Pitressin®, urine osmolarity slowly increased to approach, but rarely equal, values observed during hydropenia. Differences in plasma osmolarity in the hydrated-Pitressin® and hydropenic groups accounted to some extent for the discrepancy. When urine osmolarity was expressed as a function of plasma osmolarity, either as the osmotic U/P ratio or the urine-plasma osmotic pressure difference, values after a sufficient period of Pitressin® infusion were often comparable to those of hydropenic subjects providing solute diuresis was present. In the absence of solute diuresis, the results were not as consistent. Prolonged Pitressin® administration in several instances failed to elevate osmotic pressure differentials to values observed in hydropenic subjects.

METHODS

For the study 32 experiments were performed, 17 on eight female mongrel dogs and 15 on five male subjects. At least two experiments were usually performed on each subject, one to determine urinary concentration during hydropenia and a second to test the effectiveness of Pitressin® in concentrating the urine during hydration. All experiments were performed in the morning. The general plans of experimentation are described below.

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[2] Presented in part before the meeting of the American Physiological Society, September, 1954.

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Experiments on dogs

Previous experimental work (7) has indicated that dogs produce a more concentrated urine during hydropenia when the previous diet has been high in protein. Accordingly, in the present study, the animals were fed on a diet rich in protein (horsemeat) for two days preceding each experiment to insure conditions optimal for maximal urine concentration. If a portion of the diet was refused on either day, the experiment was not performed. All experiments were preceded by 18 hours of fasting. Urine was collected by indwelling catheter and blood either by venipuncture or from an indwelling polyethylene venous catheter. The blood was either mixed with a small amount of dry heparin or allowed to clot.

A. *Experiments on hydropenic animals:* Hydropenia was induced by 18 hours of water deprivation. In four of the experiments, the animals were anesthetized with Nembutal® (27 mg. per Kg.) administered intravenously, and in three, no anesthetic was used. Anesthesia appeared to have no influence on the results obtained. In experiments in which no solute load was administered, the procedure consisted simply in the collection of several urine specimens over intervals of 20 to 30 minutes. For experiments in which solute diuresis was induced, a priming dose of mannitol was given as a 20 per cent solution after one or two preliminary urine collections. The amount of priming dose varied; the largest dose was calculated to raise the plasma level to 32 milliosmols per L. and the smallest to 2.5 milliosmols per L. The priming dose was given over a 10 to 20-minute interval and was followed by a sustaining infusion amounting to 1.5 per cent of the priming dose per min. Following a period of time allowing for equilibration of the priming dose throughout its distribution volume, urine was collected every 10 to 20 minutes and blood specimens at appropriate intervals.

B. *Hydrated-Pitressin® experiments:* Preceding the experiments employing Pitressin®, the animals were hydrated by two loads of tap water given by stomach tube, each amounting to 5 per cent of the body weight. The first was given 16 hours and the second 45 to 60 minutes before experimentation. In 5 of the 10 experiments the animals were anesthetized with chloralose (90 mg. per Kg.) administered intravenously as a 1 per cent solution in 5 per cent glucose. Sufficient time was allowed for the absorption of the ingested water before the anesthetic was given. Experiments on animals anesthetized with chloralose did not differ in their results from the others. In experiments employing solute diuresis, hydration was maintained by the infusion of mannitol loads in large volumes of water. The volume of the priming injection amounted to 2 per cent of the body weight and of the sustaining infusion, 0.03 per cent of the body weight per minute. The concentration of mannitol in the infusions was never greater than 300 milliosmols per L. and, if less than 180 milliosmols per L., glucose was added in amounts sufficient to bring the total tonicity to this value. For experiments in which a load of mannitol was not given, a quantity of 3 per cent glucose or 1.5 per cent glucose—1.5 per cent fructose equal to 1 per cent of the body weight was infused rapidly before urine collection was started. This was followed by a continuous infusion of the same solution at the rate of 0.015 per cent of the body weight per minute. When urine collections indicated that water diuresis was approximately at its maximum, a priming injection of Pitressin®, amounting to 6.4 milliunits per kilogram contained in 4 cc. of 5 per cent glucose, was given by rapid intravenous injection and was followed by a continuous infusion of this agent at the rate of 48 mUnits per Kg. per hour contained in 30 cc. of 5 per cent glucose. Urine and blood collections were continued at appropriate intervals. Dilutions of Pitressin® were freshly prepared the morning of the experiment.

Experiments on human subjects

Preceding the experiments, the subjects were on regular diets. Breakfast was omitted the morning of experimentation. During the experiments, the subjects were recumbent, standing only to void.

A. *Experiments in the hydropenic state:* Hydropenia was induced by 15 hours of water deprivation. In experiments employing solute diuresis, the mannitol levels attained varied from 2.5 to 10 milliosmols per L. The experimental procedure did not differ essentially from that described for dogs.

B. *Hydrated-Pitressin® experiments:* The subjects were allowed water *ad libitum* the night before the experiment. In the morning just before or after arrival in the laboratory, 1,500 cc. of tap water was drunk over a 30 to 45-minute period. In the experiments in which the effect of solute diuresis was studied, a priming infusion of mannitol in 5 per cent solution was started when urine collections indicated that water diuresis was increasing. After equilibration of the priming dose and the collection of the two urine specimens during combined water and solute diuresis, a continuous intravenous infusion of Pitressin® was started. The rate of administration was 4 or 16 mUnits per kilogram per hour contained in 30 cc. of 5 per cent glucose. The collection of urine and blood specimens at regular intervals was continued. During the entire experiment, mannitol was infused as a 5 per cent solution at a rate of 1.5 per cent of the priming dose per minute. Hydration was maintained by ingestion of water in amounts approximately equal to the volumes of the urine.

In five of the experiments on hydrated subjects, inulin was included in the priming and maintenance solutions in amounts sufficient for the measurement of inulin clearance. The ampouled inulin was not subjected to alkali hydrolysis prior to use.

The freezing point of urine was determined with a Hortvet cryoscope and that for serum by means of a thermistor. Both instruments were calibrated by determining the freezing points of standard solutions of sodium chloride. The thermistor was calibrated several times during each series of determinations; the Hortvet cryoscope less frequently. The osmotic pressure of plasma

TABLE I

Effect of Pitressin® on urine flow and urine and plasma osmolarity when administered to the hydrated human subject

Period	Concurrent time min.	Urine flow cc./min./ 1.73 m²	Urine osmolarity mOsmols./ L.	Plasma osmolarity mOsmols./ L.
Experiment No. 56				
	−108 to −81	1,500 cc. tap water drunk*		
	−54 to −29	5% mannitol, 620 cc. i.v.		
	−29 to 83	5% mannitol, 9.3 cc./min. i.v.		
P-1	−26 to −13	20.1	153	284
P-2	−13 to −1	16.6	176	285
	0 to 83	Pitressin®, 4 mUnits/Kg./hr. i.v.		
1	−1 to 12	10.2	245	283
2	12 to 26	5.69	454	283
3	26 to 40	6.04	477	278
4	40 to 52	5.86	480	279
5	52 to 66	5.94	477	278
6	66 to 83	5.86	484	277
Experiment No. 121				
	−103 to −88	1,500 cc. tap water drunk*		
P-1	−58 to −33	9.24	90	
P-2	−33 to −19	11.3	59	
P-3	−19 to 3	14.5	49	
	0 to 198	Pitressin®, 16 mUnits/Kg./hr.		
1	3 to 25	1.88	242	
2	25 to 42	.846	605	281
3	42 to 70	.623	626	
4	70 to 91	.505	672	277
5	91 to 112	.468	693	
6	112 to 136	.459	739	277
7	136 to 154	.501	753	
8	154 to 178	.438	777	272
9	178 to 198	.406	787	

* Additional water was ingested during the experiments approximately equal in volume to the rate of urine flow.

and urine, expressed in milliosmols per liter, was calculated as:

$$\text{Osmolarity} = \frac{\Delta^\circ}{1.86^\circ} \times 1{,}000$$

Inulin was determined by a modification (8) of the method of Hubbard and Loomis (9).

RESULTS

Inhibition of water diuresis

Following administration of Pitressin® to the hydrated subjects, water diuresis was inhibited. Urine osmolarity promptly rose and urine flow diminished to values dependent on the amount of solute presenting for excretion. Data for typical experiments on human subjects are presented in Table I. In Experiment 56, mannitol as well as water diuresis was present prior to Pitressin® administration. Because of the magnitude of the excreted solute load, diuresis was still present after Pitressin® infusion and the urine was not highly concentrated. In Experiment 121, only a water diuresis was present prior to Pitressin® infusion. In contrast to conditions of solute loading, Pitressin® caused a marked fall in urine flow and rise in urine osmolarity. In these experiments, as in most of the others, urine became hypertonic to plasma during the second collection period after Pitressin® administration. Data for all collection periods in which the urine was hypertonic to plasma are included in subsequent figures.

Urine and plasma osmolarity in hydropenic subjects

In both hydropenic man and dog, urine osmolarity diminished with increasing solute diuresis as indicated in Figures 1 and 2. This relationship has been described previously (4–7) and has been shown to be independent of the composition of the urine or the nature of the solutes excreted. The present data indicate that at flows of less than 2 cc. per min. per m² in the dog, urine osmolarity varies widely. At higher flows in the dog and at all flows in man, the range is comparatively small and the values reproducible.

Prior to loading, the osmotic activity of plasma in hydropenic man was relatively constant and

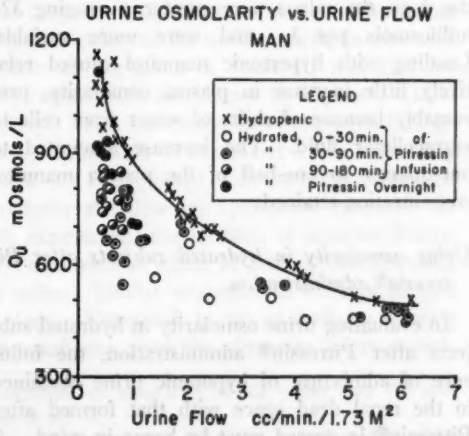

FIG. 1. URINE OSMOLARITY *versus* THE RATE OF URINE FLOW DURING HYDROPENIC AND HYDRATED-PITRESSIN® EXPERIMENTS (DATA FOR MAN)

The relation for hydropenic subjects is indicated by the hand-drawn line. Times after Pitressin® are for midpoints of urine periods.

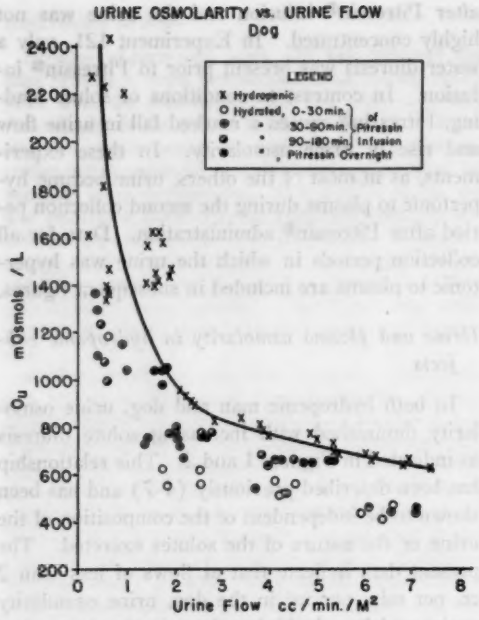

FIG. 2. URINE OSMOLARITY *versus* URINE FLOW DURING HYDROPENIC AND HYDRATED-PITRESSIN® EXPERIMENTS (DATA FOR THE DOG)

See legend Figure 1.

averaged 292 milliosmols per L. (Table II). In the dogs, the values were greater, averaging 324 milliosmols per L., and were more variable. Loading with hypertonic mannitol caused relatively little increase in plasma osmolarity, presumably because of shifts of water from cells to extracellular fluid. The increase amounted to one-quarter to one-half of the plasma mannitol concentration attained.

Urine osmolarity in hydrated subjects after Pitressin® administration

In evaluating urine osmolarity in hydrated subjects after Pitressin® administration, the influence of admixture of hypotonic urine contained in the renal dead space with that formed after Pitressin® is started must be borne in mind. A number of workers (10, 11) have given evidence that equilibrium of inflow and outflow in the renal system is not reached until thirty minutes after a change in experimental conditions. For this reason, urine collections with midpoints within thirty minutes after the start of Pitressin® infusions (Figures 1 and 2) are of doubtful physiological significance. After this interval, it would appear from the data of the experiments as given in Table I that a relatively steady state is reached.

In general, the urine osmolarities achieved after Pitressin® were, to a variable extent, less than during hydropenia. In the dog, urine osmolarity increased gradually after Pitressin® infusion but never attained values observed at comparable flows in hydropenia (Figure 1). In human subjects undergoing solute diuresis, urine osmolarity rapidly increased with Pitressin® infusion (Figure 2); values 30 to 90 minutes after start of Pitressin® were only slightly, albeit consistently, less than those observed at corresponding flows during hydropenia. On the other hand, in the absence of mannitol diuresis in man (flows usually less than 1 cc. per minute) Pitressin® was considerably less effective in producing a maximally concentrated urine. In four experiments, the highest urine osmolarity obtained after 90 to 180 minutes of Pitressin® infusion was 847 and the average peak value was 797 milliosmols per L. These values are in contrast with osmotic activities averaging 1,000 milliosmols per L. at comparable flows in hydropenic subjects.

The slow response and the failure to reach peak values with Pitressin® in the absence of solute diuresis might conceivably be explained as an artifact attributable to admixture of dilute urine contained in the dead space with the concentrated urine formed after hormone administration. This dilution effect would be of greater moment at low rates of urine flow than at the high rates of flow obtaining during solute diuresis. However, the experimental procedure and the pattern of response observed in these experiments make this

TABLE II

Average values and range of plasma osmolarity in man and dog during hydropenic and hydrated-Pitressin® experiments *

Condition	Human		Dog	
	Av.	Range	Av.	Range
Hydropenic (preloading)	292	290–294	324	314–332
Hydrated (during Pitressin®)	281	272–288	282	268–300

* Values are in mOsmols. per liter.

TABLE III

Urine flow and urine and plasma osmolarity in a hydrated subject given five pressor units of Pitressin® Tannate in Oil 15 hours previously

Period No.	Concurrent time *Min.*	Urine flow *cc./min./1.73 m²*	Urine osmolarity		Plasma osmolarity *mOsm./L.*	Osmotic U/P	
			Observed *mOsm./L.*	Expected* *mOsm./L.*		Observed	Expected*
Experiment 146							
Disc	— to 0	—	790			2.83	
1	0 to 25	.54	809	1,017	279	2.90	3.45
2	25 to 50	.90	801	905	275	2.91	3.10
	54 Aqueous Pitressin®, 35 mUnits i.v.						
	54 to 117 Aqueous Pitressin®, 4 mUnits/Kg./hr. i.v.						
3	50 to 77	1.09	716	875	275	2.60	3.00
4	77 to 98	1.25	669	847	274	2.44	2.91
5	98 to 117	1.00	723	892	274	2.64	3.05

* Values for corresponding rates of urine flow in hydropenic subjects (from relationship expressed by lines in Figures 1 and 3).

explanation unlikely. In Experiment 121 (Table I), for example, urine was voided at approximately 20-minute intervals, thus reducing the effect of bladder dead space. Equilibrium conditions appear to be established 40 to 60 minutes after the start of Pitressin® infusion. Thereafter urine flow showed relatively little change and urine osmolarity gradually increased at a rate of about 1 milliosmol per L. per minute. In this experiment, specimens obtained after three hours of Pitressin® infusion, during which time the subject had voided eight times, had osmolarities considerably less than observed in the hydropenic subject.[5] The procedure and data of the other three experiments of this type were essentially similar.

It would appear from the gradual increase in urine osmolarity with time during infusion of Pitressin® that eventually hydropenic values would be achieved. To test this possibility several experiments were performed on hydrated subjects in which Pitressin® was present in the circulating fluids for 15 hours before experimentation. The human subjects were given 3 to 5 units of Pitressin® Tannate in Oil intramuscularly at a time when normally hydrated the afternoon preceding the experiment. During and after the evening meal they drank 2,500 cc. of water. The values for urines collected the next morning in one such experiment are given in Table III and data for both experiments are shown in Figure 1 (solid circles). With the exception of one period (Figure 1), urine was of somewhat lower osmolarity than would be predicted from the relation between osmolarity and flow for hydropenic subjects. In both experiments the infusion of aqueous Pitressin® was without effect in further concentrating the urine. Similar experiments were performed on dogs. Pitressin® in oil as well as aqueous Pitressin® was given the afternoon before experi-

[5] It is obvious that under conditions of Experiment 121, the urine formed during a given period will be of greater concentration than that voided. The osmolarity of the urine formed may be calculated by a simple mixing equation, assuming the new formed urine to mix completely with dead space urine and that it is formed at a rate equal to the observed rate of urine flow. The equation is based on the identity

Number of solutes in urinary tract before mixing = Number of solutes in urinary tract after mixing

or

$$(V_{DS}O_{u_1}) + (VO_{u_e}) = O_{u_2}(V + V_{DS})$$

in which V_{DS} represents the dead space volume, O_{u_1}, the osmolarity of urine last voided (assumed osmolarity of dead space urine at start of period), O_{u_e}, the osmolarity of urine formed during period, V, the volume of urine formed during period (volume voided), and O_{u_2}, the osmolarity of urine voided at end of period.

Applying this equation to the last three periods of Experiment 121 and assuming a dead space volume of 30 cc. the osmolarity of the urine formed (O_{u_e}) was found to average 45 milliosmols per L. greater than that voided. Thus urine formed during period 9 would have, by these assumptions, an osmolarity of 822 rather than 777 milliosmols per L.

Solving the equation for V_{DS}, assuming that urine formed during the last three periods was concentrated to 1,000 milliosmols per L. (as would be predicted from hydropenic values), the calculation indicates that the urine formed would have to mix with dead space volumes of 110 to 180 cc. to account for the osmolarities observed.

mentation together with water to the amount of 5 per cent of the body weight by stomach tube. The next morning a second load of water equal in amount to the first was given and the experiment performed with aqueous Pitressin® infusion in the usual manner. In both of these experiments (Figure 2, solid circles), urine osmolarity was less than at comparable flows during hydropenia.

The two rates of Pitressin® infusion employed in the human subjects, 4 and 16 mUnits per Kg. per hr., did not differ significantly in their effects on urine osmolarity.

Interrelationships between urine and plasma osmolarity

Plasma osmolarity was in all cases lower in the hydrated than in the hydropenic subjects (Table II). The average difference in dogs amounted to 42 milliosmols per L. and in man to 11 milliosmols per L. It seemed possible that the lower urine osmolarity after Pitressin® might be in part related to the dilution of body fluids in the hydrated subjects. Accordingly, several expressions relating urine and plasma osmolarity were investigated as a means of further assessing concentrating ability after Pitressin®. These included the osmotic U/P ratios (O_u/O_p), the osmotic pressure differences of urine and plasma (O_u–O_p), and the water economy.

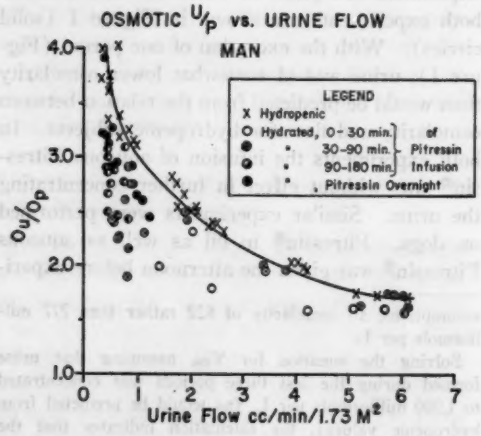

FIG. 3. OSMOTIC U/P RATIO *versus* URINE FLOW (DATA FOR MAN)

See legend Figure 1.

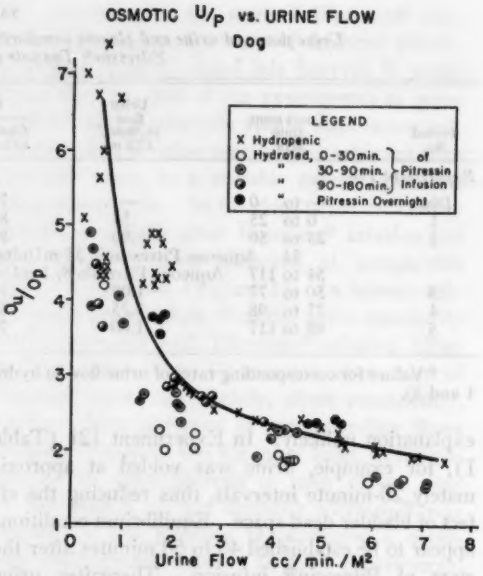

FIG. 4. OSMOTIC U/P RATIO *versus* URINE FLOW (DATA FOR THE DOG)

See legend Figure 1.

The relation between osmotic U/P ratios and urine flow for man and dog are shown in Figures 3 and 4, respectively. In man, the relative positions of hydropenic and hydrated-Pitressin® points were not significantly altered by expressing the data as the osmotic U/P ratio as would be predicted from the small differences in plasma osmolarity of hydrated and hydropenic subjects. Under conditions of solute diuresis, urines collected within 30 to 90 minutes of Pitressin® infusion gave osmotic U/P ratios differing little from hydropenic values. In the absence of solute diuresis, the agreement between hydropenic and hydrated Pitressin® values was also improved. Several specimens collected after Pitressin® had been infused for 90 to 180 minutes or had been present in circulating fluids overnight had osmotic U/P ratios comparable to hydropenic values. On other occasions, after Pitressin® had been present for similar periods, the ratios were significantly less than would be predicted from hydropenic values (Table I, Experiment 121, and Table III).

In the dog, expressing urine osmolarity as the osmotic U/P ratio (Figure 4) resulted in much better agreement of hydropenic and hydrated-

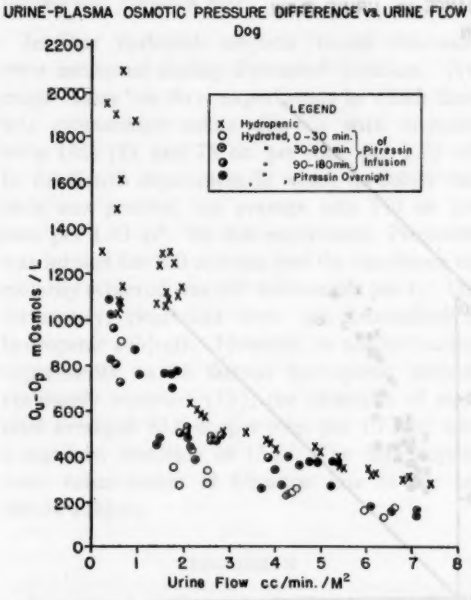

FIG. 5. THE URINE-PLASMA OSMOTIC PRESSURE DIFFERENCE PLOTTED *versus* THE RATE OF URINE FLOW (DATA FOR THE DOG)

Pitressin® values than was the case for the plot of urine osmolarity *versus* urine flow (Figure 2). After 90 minutes of Pitressin® infusion, and in some instances after a shorter interval, the osmotic U/P ratios were clearly comparable to those observed in hydropenia. As for the experiments in man, in the absence of solute diuresis, osmotic U/P ratios after Pitressin® tended to fall short of the high values observed in hydropenic animals but the scatter in this region makes assessment of the data difficult.

The osmotic activity differences between urine and plasma (O_u–O_p) in hydrated dogs (Figure 5), were in agreement with the hydropenic values only after overnight Pitressin® administration. With shorter periods of Pitressin® infusion, values of O_u–O_p tended to be lower than in the hydropenic animals. For man, the plot of O_u–O_p *vs.* urine flow showed agreement between hydrated and hydropenic values similar to that for osmotic U/P ratios (Figure 3) and is not shown.

The rate of water economy (7), termed the negative free water clearance or T^cH_2O by other workers (12), was also investigated in comparing data for hydrated-Pitressin® and hydropenic experiments. Water economy was calculated as the difference between the solute, or osmolar clearance and rate of urine flow[6] by the expression—

$$H_2O_{Eo} = \frac{O_u}{O_p} V - V$$

in which O_u and O_p are the osmolarities of urine and plasma respectively and V, the rate of urine flow. Because water economy is a function of the osmotic U/P ratio and urine flow, plots comparing water economy and urine flow show the same relation for hydrated-Pitressin® and hydropenic experiments as observed in plots of osmotic U/P ratio *vs.* urine flow. Data pertaining to water economy in man are shown in Figure 6. The graph is similar to that employed by others (13, 14) to assess the renal concentrating function. The solute (osmolar) clearance $\frac{O_u V}{O_p}$, has been plotted on the ordinate *vs.* urine flow on the abscissa. The diagonal line, termed the isosmotic parameter, indicates the relation between these variables which would have been observed had tubular fluid been unaffected by the concentrating mechanism and remained isosmotic with plasma. The horizontal distance from any point to the isosmotic parameter thus indicates the rate of economy of water resulting from the concentrating operation. It may be seen that at urine flows greater than 1.0 cc. per min., 30 to 90 minutes of Pitressin® infusion resulted in rates of water economy about equal to those observed in hydropenic subjects. On the other hand, at low rates of urine flow, shown in detail in the inset, hydropenic rates of water economy were reproduced by Pitressin® in some experiments; in others performed in an identical manner, the values were less than would be expected.

The effectiveness of Pitressin® in maintaining maximal urine osmolarity after hydration of the hydropenic subject

In view of the failure of Pitressin® to concentrate the urine maximally in the absence of solute diuresis in several experiments, it was of

[6] The water economy is the rate at which water must be reabsorbed to produce a concentrated urine, assuming reabsorption to occur from a tubular fluid isosmotic with plasma containing only the solutes to be excreted.

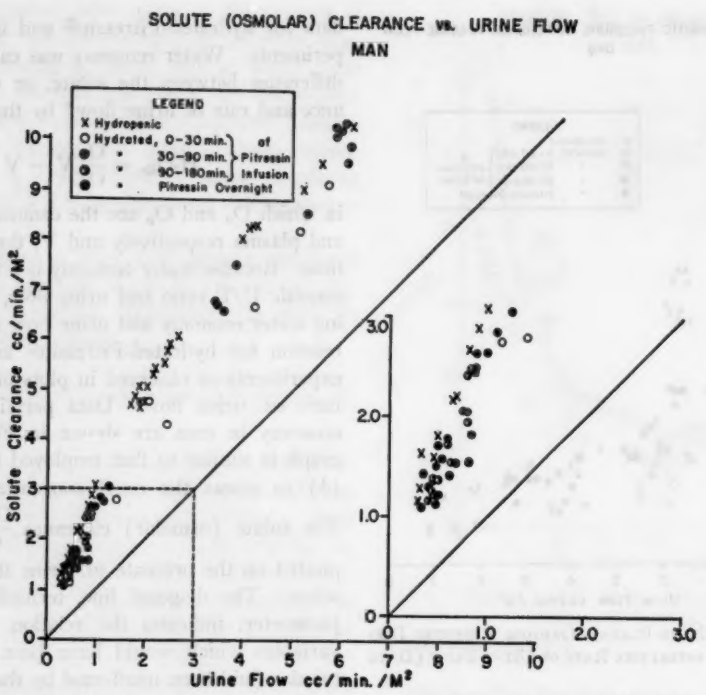

FIG. 6. THE SOLUTE CLEARANCE *versus* URINE FLOW (DATA FOR MAN)
The region within the dotted lines is shown in detail in the inset at right.

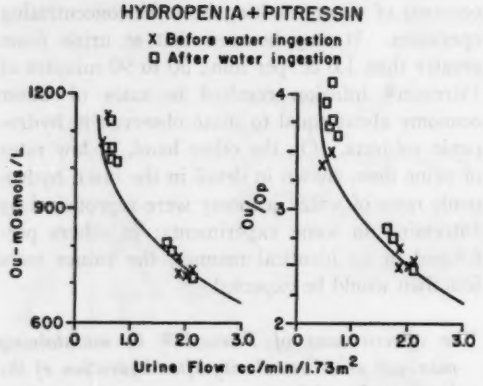

FIG. 7. URINE OSMOLARITY *versus* URINE FLOW (LEFT) AND OSMOTIC U/P RATIO *versus* URINE FLOW (RIGHT) BEFORE AND AFTER WATER INGESTION BY TWO HYDROPENIC SUBJECTS RECEIVING PITRESSIN®

In the experiment in which points group about urine flows of 2 cc. per minute, mild mannitol diuresis was present. In this experiment plasma osmolarity fell from 293 to 285 mOsmols. per L. with water ingestion; in the other, the fall was from 286 to 272.

interest to determine whether in the hydropenic subject Pitressin® would keep urine osmolarity at a high level after water ingestion. Two experiments of this type were performed. In one, the subject was undergoing mild solute diuresis with mannitol throughout; in the other, there was no solute diuresis. After several urines were collected while the subject was hydropenic and receiving Pitressin® at rates of 16 mUnits per Kg. per hr., 1500 cc. of water were ingested and the Pitressin® infusion continued. In one experiment, urines were collected for one hour after hydration and in the other for two hours. The results are indicated in Figure 7. In neither experiment was there any significant deviation from hydropenic values after hydration, either in urine osmolarity or in osmotic U/P ratio. Thus, it appears that Pitressin® will keep the urine at maximal osmolarity when the hydropenic subject becomes hydrated but may fail to produce a maximally concentrated urine when administered to the hydrated subject undergoing water diuresis.

Glomerular filtration rate

In four hydrated subjects, inulin clearances were measured during Pitressin® infusion. Average values [7] in three experiments in which there was concomitant solute diuresis with mannitol were 101, 111 and 78 cc. per min. per 1.73 m^2. In the fourth experiment in which no solute diuresis was present, the average was 140 cc. per min. per 1.73 m^2. In this experiment, Pitressin® was infused for 100 minutes and the maximum osmolarity achieved was 847 milliosmols per L. Unfortunately, clearances were not determined in hydropenic subjects. However, in similar loading experiments on 15 normal hydropenic subjects previously reported (15), the clearance of mannitol averaged 83.6 cc. per min. per 1.73 m^2 with a standard deviation of 15.2. The data suggest some enhancement of filtration rate in the hydrated subject.

DISCUSSION

Assessment of the present data as regards the ability of Pitressin® to concentrate urine rests in part on the nature of the concentrating mechanism and the means of measuring its activity. Page and Reem (16) from studies on the dog, and Zak, Brun, and Smith (14) from studies on man, have shown that at high rates of urine flow, attained by solute loading in hydropenic subjects, the rate of abstraction of solute free water from tubular fluid, or the water economy, tends to reach a constant maximal volume. Although in about one-half of the human subjects examined, this quantity demonstrated some systematic variation with changes in urine flow, its magnitude was nevertheless regarded as a fixed renal function. For its demonstration, the solute clearance (measured approximately by the rate of solute excretion) must be high so that a large quantity of water reaches the concentrating mechanism. At lower rates of solute clearance, approximating those of the present study, the rate of water abstraction by the concentrating mechanism diminishes. Under these conditions, the authors state that maximal urinary concentration is dependent on a limiting osmotic U/P ratio.

[7] Averages are for three or four urine collection periods. Values obtained within 30 minutes of the start of Pitressin® infusion are not included in the averages.

Assuming from these interpretations that under the conditions of the present study, the osmotic U/P ratio is a direct measure of the activity of the concentrating mechanism, it may be said that with solute diuresis in man and in the dog, Pitressin® acts as a maximal stimulus to the concentrating mechanism, provided it is infused over an interval of approximately thirty minutes or longer in man and ninety minutes in the dog. This interpretation is in accord with the studies of human subjects reported by Zak, Brun, and Smith (14).

A similar conclusion would be reached if the urine-plasma osmotic pressure difference were taken as a measure of the activity of the concentrating mechanism. Using this standard of reference, the sole difference in the interpretation would be that in the dog, Pitressin® must be present in circulating fluids for twelve hours or more before the concentrating mechanism is maximally stimulated. As a fundamental measure of the renal concentrating operation, the present study cannot discriminate among the osmotic U/P ratio, the urine-plasma osmotic pressure difference, and the water economy.

In the absence of solute diuresis, the results with Pitressin® are less consistent and more difficult to interpret. When administered to the hydrated subject undergoing water diuresis, urine osmolarity increased, at first rapidly and then more slowly. In only one experiment did the osmotic differentials attained come within the range of hydropenic subjects. Similar results were obtained after Pitressin® had been present in circulating fluids overnight. On the other hand, once maximal urine osmolarity is achieved in the hydropenic subject, it appears to be maintained by Pitressin® despite water ingestion. The latter result confirms the observations of Sodeman and Engelhardt (1) and agrees with data given in a recent study by Leaf, Bartter, Santos, and Wrong (17).

An artifact in these observations seems unlikely. It is, of course, possible, as mentioned previously, that in the experiments in which water diuresis preceded Pitressin® infusion, hypertonic urine formed after Pitressin® was given was contaminated with residual hypotonic urine present in the renal dead space. However, the conduct of the experiments with frequent voidings and the data

indicating the attainment of relatively steady state makes such an artifact seem unlikely. One conclusion which seems obvious is that with Pitressin®, ceiling values for urine-plasma osmotic differentials are achieved much more slowly at normal rates of solute excretion than under conditions of solute diuresis. The reason for this is not clear. It is conceivable that the high urine-plasma osmotic differentials characteristic of low rates of urine flow require greater activity of the concentrating mechanism for their production than the lower differentials (or the tubular maximum for water abstraction) present during solute diuresis.[8] If such is the case, the ability to attain high values at low rates of flow might then constitute a test of the concentrating mechanism more sensitive than that correlating urine osmolarity with urine flow during solute diuresis (15). The results give no evidence for and, in fact, make doubtful the assumption that a greater blood concentration of ADH is needed to induce maximum anti-diuresis when the rate of solute excretion is high than when it is in the usual range (19).

The experiments in which Pitressin® was present in circulating fluid overnight give only meager support to a conclusion that might be drawn from the data of the acute experiments, namely, that with prolonged Pitressin® infusion maximal osmotic differentials would always be achieved. After Pitressin® had been present for 15 hours, maximal values were observed for a few periods in one experiment. In the other, the values were less than predicted. In these experiments there was no possibility of contamination with hypotonic dead space urine. The possibility that the blood concentrations of Pitressin® were too low to produce maximum osmotic differentials seems unlikely from the fact that infusion of the aqueous material produced no increase in concentration. The results in general confirm the more extensive studies by Taylor, Peirce, and Page (3) in which specific gravities were employed to measure urinary concentrations. As was pointed out by these authors, not only may the urinary concentrations be less after Pitressin® than during hydropenia, but also the results are not consistent even when the agent is given over prolonged periods. Because of the inconsistencies, use of Pitressin® as a substitute for water deprivation in clinically testing the concentrating function of the kidney would appear to be of limited value.

A factor which should not be overlooked as contributing to the generally lower and inconsistent osmotic differentials in the absence of solute diuresis is the hydration of the subjects receiving Pitressin®. There are a number of ways by which hydration could conceivably influence the concentrating mechanism. Dilution of body fluids might diminish the concentrations of certain intra- and/or extracellular solutes, critical for maximal activity of the concentrating mechanism. The possibility that hydration might variably stimulate secretion of adrenal cortical hormones antagonistic to Pitressin®, evidence for which has recently been reviewed (20), should also be considered. Similarly, substances with anti-diuretic activity other than ADH might be present in the hydropenic subject, supplementing the action of the pituitary hormone and disappearing with hydration. Finally, differences in filtration rate in the hydrated and hydropenic subjects might be responsible for the differences in urinary concentration.

Data in the literature, however, appear to indicate that hydration is not a significant factor responsible for the lower and inconsistent urinary concentrations after Pitressin®. In the experiments by Taylor, Peirce, and Page (3), no attempt was made to hydrate the patients during Pitressin® administration, yet the urinary concentrations measured by specific gravities were often lower than after water deprivation. Perhaps more germane are observations made on patients with diabetes insipidus. Studies by Brodsky and Rapoport (21) suggest that even when hydropenic, patients with this disease while receiving Pitressin® fail to produce a maximally concentrated urine at low rates of urine flow. Subsequent examination, in this laboratory, of two additional patients with diabetes insipidus have confirmed these observations. On the other hand,

[8] It should be noted that this assumption is not in accord with thermodynamic calculations of the theoretical, minimal energy required for the production of a concentrated urine. When "distal osmotic" work is calculated as described in a previous paper (18), by substituting in the equation of von Rohrer values for urine and plasma osmolarity, the minimal energy requirement is found to be relatively small for highly concentrated urine at low rates of flow as compared to conditions of mild solute diuresis.

data given by Leaf, Mamby, Rasmussen, and Marasco (22) would suggest that maximal urine osmolarity may occasionally be achieved by these subjects. It is of interest that under conditions of solute diuresis, subjects with diabetes insipidus given Pitressin® and rendered hydropenic are able to produce urine comparable in concentration to that of normal individuals (21). Their behavior under both loading and non-loading conditions thus appears to correspond closely to that of the hydrated normal subject given Pitressin®

Data on glomerular filtration rate are too scanty in the present study to establish a relationship between this function and urinary concentration. The observations do suggest that filtration rate was greater in the hydrated than in hydropenic subjects, a trend which has been frequently reported. Reports by other workers on the influence of filtration on urinary concentration allow some prediction as to the effect of this function in the present study. Platt (23) has pointed out the importance of considering the osmolarity-flow relationship in terms of nephron flow. When a large fraction of the renal tissue is removed in the experimental animal, renal concentrating ability is reduced although the nephrons remaining are presumably not injured. Since the partially nephrectomized animal has daily rates of solute excretion differing little from the normal, it is apparent that in terms of the nephrons remaining, a solute diuresis is occurring, a condition in which a reduced urine osmolarity would be predicted. This observation points out that the osmolarity-flow relationship would be more precisely expressed in terms of flow per nephron rather than total urine flow. The relationship observed with total flow is fairly well defined because a significant change in the number of functioning nephrons probably does not occur with solute diuresis (24) so that average flow per nephron is a relatively fixed fraction of total flow. Applying this concept to the hydrated-Pitressin® experiments, if activation of previously inactive nephrons accounted for the enhancement of filtration rate, average flow per nephron would be a smaller fraction of a given total flow and the osmolarity-flow relationship would be shifted to the right, *i.e.*, urine osmolarity would be greater at a given flow. Alternatively, if the increase in filtration rate occurs, as commonly believed, without change in the number of functioning nephrons, flow per nephron as a fraction of total flow would be the same as in hydropenic subjects and the osmolarity-flow relationship would be unchanged. This prediction has experimental verification in the studies of Thompson and Barrett (25). Moderate reduction in filtration rate in dogs undergoing mannitol diuresis by inflation of a balloon catheter in the aorta had no effect on the osmolarity-flow relationship. Marked reduction, presumably accompanied by a diminished number of functioning nephrons, resulted in a lower osmolarity than in the normal animal. The observation suggests that under relatively normal conditions, alterations in filtration rate unaccompanied by a change in the number of functioning nephrons will not affect the osmolarity-flow relationship.

SUMMARY

Administration of Pitressin® in supramaximal amounts to hydrated man and dog resulted in urine osmolarities somewhat less than in hydropenic subjects at comparable rates of urine flow. The discrepancy was reduced when the lower plasma osmolarity of the hydrated subject was taken into account. When the data obtained under conditions of solute diuresis were expressed in terms of the osmotic U/P ratio or the urine-plasma osmotic pressure difference, values for hydropenic and hydrated-Pitressin® conditions were comparable.

In the absence of solute diuresis, particularly in man, less consistent results were obtained. The urine-plasma osmotic differentials approached hydropenic values much more slowly than when Pitressin® was administered during solute diuresis and in several experiments in which Pitressin® was present in circulating fluids for prolonged periods, maximal values were never attained. On the other hand, water ingestion by the hydropenic subject receiving Pitressin® did not lower the osmotic differential. The reason for these inconsistencies is not known.

ACKNOWLEDGMENT

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REFERENCES

1. Sodeman, W. A., and Engelhardt, H. T., A renal concentration test employing posterior pituitary extract. Am. J. M. Sc., 1942, **203**, 812.
2. Little, J. M., Wallace, S. L., Whatley, E. C., and Anderson, G. A., Effect of Pitressin on the urinary excretion of chloride and water in the human. Am. J. Physiol., 1947, **151**, 174.
3. Taylor, R. D., Peirce, J. D., and Page, I. H., Use of posterior pituitary extract in tests of urinary concentration. Am. J. M. Sc., 1945, **209**, 235.
4. McCance, R. A., The excretion of urea, salts and water during periods of hydropaenia in man. J. Physiol., 1945, **104**, 196.
5. Hervey, G. R., McCance, R. A., and Tayler, R. G. O., Further observations on the causes of a diuresis during hydropenia. J. Physiol., 1946, **104**, 43P.
6. Rapoport, S., Brodsky, W. A., West, C. D., and Mackler, B., Urinary flow and excretion of solutes during osmotic diuresis in hydropenic man. Am. J. Physiol., 1949, **156**, 433.
7. West, C. D., and Rapoport, S., Urine flow and solute excretion of hydropenic dog under "resting" conditions and during osmotic diuresis. Am. J. Physiol., 1950, **163**, 159.
8. West, C. D., and Rapoport, S., Method for determination of sucrose and sorbose in blood and urine. Proc. Soc. Exper. Biol. & Med., 1949, **70**, 140.
9. Hubbard, R. S., and Loomis, T. A., The determination of inulin. J. Biol. Chem., 1942, **145**, 641.
10. Michie, A. J., and Michie, C. R., Attainment of equilibrium between plasma and urine, with reference to measurement of renal clearances. J. Urol., 1951, **66**, 518.
11. Bradley, S. E., Nickel, J. F., and Leifer, E., The distribution of nephron function in man. Tr. A. Am. Physicians, 1952, **65**, 147.
12. Wesson, L. G., Jr., and Anslow, W. P., Jr., Effect of osmotic and mercurial diuresis on simultaneous water diuresis. Am. J. Physiol., 1952, **170**, 255.
13. Smith, H. W., Renal excretion of sodium and water. Federation Proc., 1952, **11**, 701.
14. Zak, G. A., Brun, C., and Smith, H. W., The mechanism of formation of osmotically concentrated urine during the antidiuretic state. J. Clin. Invest., 1954, **33**, 1064.
15. Brodsky, W. A., Rapoport, S., Graubarth, H. N., and Levkoff, A. H., Osmotic diuresis as a measurement of renal function in man. J. Applied Physiol., 1952, **5**, 62.
16. Page, L. B., and Reem, G. H., Urinary concentrating mechanism in the dog. Am. J. Physiol., 1952, **171**, 572.
17. Leaf, A., Bartter, F. C., Santos, R. F., and Wrong, O., Evidence in man that urinary electrolyte loss induced by Pitressin is a function of water retention. J. Clin. Invest., 1953, **32**, 868.
18. Rapoport, S., West., C. D., and Brodsky, W. A., Excretion of solutes and osmotic work during osmotic diuresis of hydropenic man. The ideal and the proximal and distal tubular work; the biological maximum of work. Am. J. Physiol., 1949, **157**, 363.
19. Lauson, H. D., The problem of estimating the rate of secretion of antidiuretic hormone in man. Am. J. Med., 1951, **11**, 135.
20. Gaunt, R., Birnie, J. H., and Eversole, W. J., Adrenal cortex and water metabolism. Physiol. Rev., 1949, **29**, 281.
21. Brodsky, W. A., and Rapoport, S., The mechanism of polyuria of diabetes insipidus in man. The effect of osmotic loading. J. Clin. Invest., 1951, **30**, 282.
22. Leaf, A., Mamby, A. R., Rasmussen, H., and Marasco, J. P., Some hormonal aspects of water excretion in man. J. Clin. Invest., 1952, **31**, 914.
23. Platt, R., Renal failure. Lancet, 1951, **1**, 1239.
24. West, C. D., and Rapoport, S., Glomerular filtration rate and renal plasma flow during mannitol loading in hydropenic dogs. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 716.
25. Thompson, D. D., and Barrett, M. J., Urine flow and solute excretion during osmotic diuresis. Am. J. Physiol., 1954, **176**, 33.

THE EFFECT OF INTRAVENOUSLY-ADMINISTERED ACTH ON PLASMA 17, 21-DIHYDROXY-20-KETOSTEROIDS IN NORMAL INDIVIDUALS AND IN PATIENTS WITH DISORDERS OF THE ADRENAL CORTEX[1]

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The response of the adrenal cortex to exogenous ACTH has been recognized as one of the more specific and quantitative tests of adrenal function (1). Changes in the 24-hour excretion values of urinary 17-ketosteroids and 17-hydroxycorticosteroids during ACTH administration have proved to be valuable indices of adrenocortical capacity (2, 3). With the development of technics for the chemical estimation of free 17, 21-dihydroxy-20-ketosteroids in plasma (4, 5), more direct measurement of adrenal activity has become possible. Recent investigations have demonstrated the usefulness of the plasma 17-hydroxycorticosteroid response to ACTH in assessing adrenal function in normal individuals and in certain adrenocortical disorders (6–11).

Studies have been performed in this laboratory on plasma 17-hydroxycorticosteroid levels in patients with endocrine and non-endocrine diseases (12). During the course of these investigations, it became apparent that there are disease states, particularly primary adrenal insufficiency and hypopituitarism, in which plasma 17-hydroxycorticosteroid levels determined before and after ACTH administration may give a more accurate picture of adrenal capacity than single plasma 17-hydroxycorticosteroid levels. These findings prompted an evaluation of plasma 17-hydroxycorticosteroid response to ACTH in patients with a variety of disorders of the adrenal cortex as compared with normal subjects.

METHODS AND MATERIALS

1. Plasma 17, 21-dihydroxy-20-ketosteroids were determined by the modified Silber-Porter method (5) as previously described (12). Normal values in this laboratory range from 4 to 32 micrograms per 100 ml. plasma (12).

2. The intravenous route of ACTH administration was selected as the most direct method of adrenal stimulation, since it avoids the variability of absorption and local tissue inactivation which have been reported to occur when ACTH is injected intramuscularly (2).

Because of the contradictory reports in the literature concerning optimal dosage and duration of administration of ACTH, it appeared impractical to aim for maximal adrenocortical stimulation or for the attainment of a steady state of steroid output (2, 11, 13). It seemed more informative to adhere to a standardized method of adrenocortical stimulation, and to compare the response of patients with disordered adrenal function with that of normal individuals under these standard conditions. The technic of the ACTH test used was as follows:

Twenty-five mgm. (25 i.u.) of ACTH (Armour ACTHAR-C, Lot R-K23601)[4] freshly dissolved in 500 ml. of 5 per cent dextrose and water was administered at a constant rate over a period of four hours. Twenty-five ml. blood specimens to which heparin[5] was added were drawn at the beginning and end of the ACTH infusion. Specimens were centrifuged, and 10 ml. plasma immediately separated and refrigerated. 17, 21-dihydroxy-20-ketosteroid determinations were performed within 72 hours. (In a few instances indicated in the Tables where the intravenous route could not be used ACTH was given by intramuscular injection.)

CLINICAL MATERIAL

1. *Normal individuals.*

2. *Addison's disease.*

3. *Hypopituitarism.* In six of the ten patients in this group there was definite roentgenographic or operative evidence of pituitary chromophobe adenoma or of craniopharyngioma, in addition to clinical findings characteristic of hypopituitarism. One patient had a suprasellar tumor, probably a glioma of the optic chiasm. In one case, the major portion of the pituitary had been removed or destroyed during craniotomy twelve years prior to the study. One case represented an example of postpartum necrosis of the pituitary. In the tenth patient,

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[4] ACTH used in these studies was generously supplied by Armour & Co.

[5] Heparin was supplied by Dr. Kenneth Thompson of Organon, Inc.

who had no demonstrable intracranial lesion, the diagnosis was based on clinical hypogonadism, hypoadrenalism, and hypothyroidism. The fact that the hypothyroidism was secondary was established by low serum precipitable iodine levels which rose to normal following the administration of thyroid-stimulating hormone.

4. *Cushing's syndrome.* In the three patients with hyperadrenalism due to bilateral adrenocortical hyperplasia, diagnosis was based on clinical and laboratory findings, and confirmed by operation and histologic study of the adrenals. In the single patient with adrenal carcinoma,[6] the clinical features suggesting malignancy were 1) a "mixed" clinical picture (*i.e.*, evidence of hypersecretion of both 11, 17-oxysteroids and of androgens), 2) very high urinary 17-ketosteroid and 17-OH-corticosteroid values, 3) an 8 cm. unilateral suprarenal mass, and 4) numerous metastatic nodules in the liver (by palpation) and lungs (by X-ray).

5. *Congenital adrenal hyperplasia.* Of the six patients studied, four were female pseudohermaphrodites, and two were males with macrogenitosomia praecox. All patients in this group excreted increased amounts of urinary 17-ketosteroids, which fell to normal levels following cortisone administration. None of the patients showed evidence of adrenal insufficiency, and none had the "salt-losing" form of the disorder.

[6] Studies in this patient were carried out through the courtesy of Dr. Herbert Koteen of the New York Hospital.

RESULTS

The results are summarized graphically in Figure 1.

A. *Normal subjects*

Four-hour intravenous ACTH tests were performed in eleven normal individuals ranging in

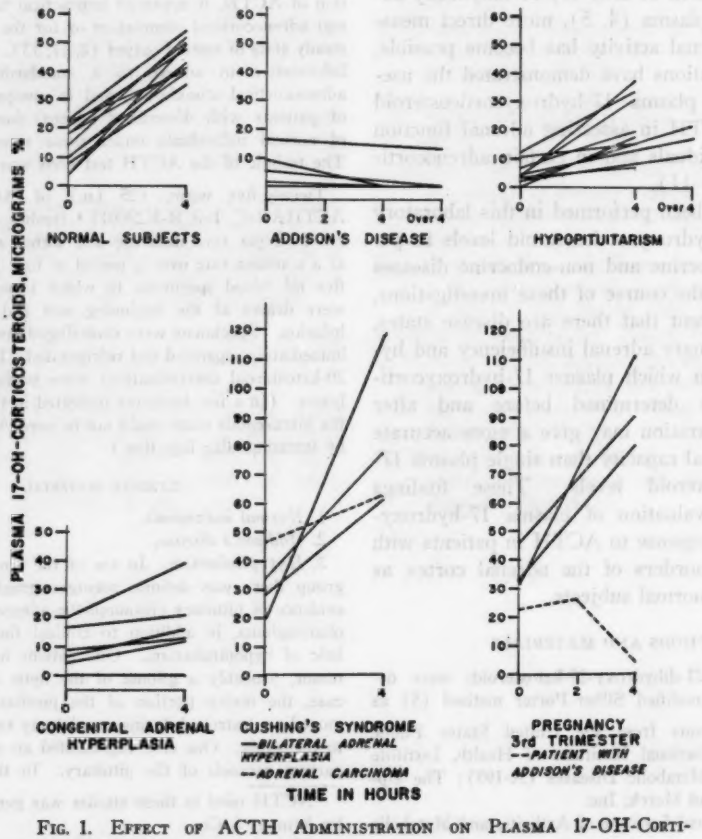

FIG. 1. EFFECT OF ACTH ADMINISTRATION ON PLASMA 17-OH-CORTICOSTEROID LEVELS IN NORMAL INDIVIDUALS AND IN PATIENTS WITH ABNORMAL ADRENOCORTICAL FUNCTION

TABLE I

Effect of 25 mgm. ACTH administered intravenously over four hours on plasma 17-OH-corticosteroid levels of normal subjects

Patient	Sex	Plasma 17-OH-corticosteroids, *micrograms* %	
		Before ACTH	After ACTH
1	♂	22	40
2	♂	4	49
3	♂	21	48
4	♀	10	39
5	♀	18	41
6	♀	8	35
7	♀	9	42
8	♀	23	54
9	♀	14	52
10	♀	18	48
11	♀	4	42

age from 14 to 59 years. The range of control values of 17, 21-dihydroxy-20-ketosteroids was 4 to 23 micrograms per 100 ml. of plasma. At the end of the ACTH infusion, values ranged from 35 to 54 micrograms per cent. There was no apparent correlation between control values and levels attained after ACTH. For example, as can be seen in Table I, patients No. 2 and No. 3 attained nearly identical plasma 17-hydroxycorticosteroid levels, despite the widely different control values (4 and 21 micrograms per cent, respectively.)

B. *Primary adrenal insufficiency*

Seven ACTH tests were performed in as many patients with documented Addison's disease. In none of these patients was a rise in plasma 17-hydroxycorticosteroid levels seen after ACTH administration (see Figure 1 and Table II). In four cases (patients J. B., F. K., C. H., and R. F.), the control values of plasma 17-hydroxycorticosteroids were within the normal range (12). However, the response of all four patients to ACTH administration was distinctly abnormal in that post-ACTH levels of plasma 17-hydroxycorticosteroids failed to show an increase over control values. The following is a brief summary of the findings in one of these four patients.

TABLE II

Effect of ACTH on plasma 17-OH-corticosteroid levels in patients with Addison's disease

Patient	Sex	Total dose *mgm.*	Duration of administration	Route of administration	17-OH-corticosteroids, *micrograms* %	
					Before ACTH	After ACTH
R. L.	♀	25	4 hrs.	Intravenous	1	0
J. B.	♂	25	4 hrs.	Intravenous	4	5
L. B.	♂	25	2 hrs.	Intravenous	0	0
F. K.	♀	25	4 hrs.	Intravenous	9	0
R. F.	♂	50	4 hrs.†	Intravenous	16	13
R. G.	♀	300	3 days	Intramuscular	1	0
C. H.	♂	300	3 days	Intramuscular	5	0
M. K.*	♀	25	4 hrs.	Intravenous	23	4

* Pregnant Addisonian in the third trimester.
† Two four-hr. infusions administered on successive days.

A 13-year old white boy (R. F.) had had numerous bouts of nausea and vomiting following minor respiratory infections since the age of 6. He had sustained two severe episodes of hypotension, one of which was followed by a prolonged period of nitrogen retention. When first seen in Babies Hospital, he was diffusely tanned, with one deeply pigmented scar. Blood pressure was 90/60, urinary corticoids[7] were 0.4 to 1.9 mgm. per 24 hours, and serum sodium was 128 mEq. per liter. Four-hour intravenous ACTH tests were performed on two successive days. The control plasma 17-hydroxycorticosteroid level was 16 micrograms per cent (see Table II). After the second ACTH infusion, the level was 13 micrograms per cent. On a regimen of added salt, DOC trimethylacetate, and cortisone, the patient has gained weight and felt well. Repeated serum sodium determinations have been normal.

The eighth patient, M. K., a woman with Addison's disease who was in the third trimester of pregnancy when studies were performed, had a control plasma 17-hydroxycorticosteroid value of 23 micrograms per cent. After two hours of intravenous ACTH infusion, her plasma level was 26 micrograms per cent (not considered a significant rise), and at the fourth hour, the level was 4 micrograms per cent.[8]

C. *Hypopituitarism*

The effect of ACTH administration was studied in ten patients with hypopituitarism (see Table III). Control plasma 17-hydroxycorticosteroid levels were low, ranging from 0 to 12 micrograms per cent. After ACTH the levels attained by most of these patients were considerably lower than those found in ACTH-stimulated normal individuals (compare Tables I and III). How-

[7] Total urinary corticoids determined by the Porter-Silber reaction after hydrolysis with beta-glucuronidase. Normal values, 3 to 9 mgm. per 24 hours.

[8] This low value may represent a technical error since the optical density of the blank was unusually high.

TABLE III

Effect of ACTH on plasma 17-OH-corticosteroids in patients with hypopituitarism *

Patient	Sex	Lesion	Dose ACTH mgm.	Plasma 17-OH-corticosteroids gamma % Before ACTH	After ACTH
J. M.	♂	Idiopathic	475†	4	17
R. H.	♀	? Postpartum pituitary necrosis	25	4	13
T. R.	♂	Post-hypophysectomy	a) 50‡	2	11
			b) 25§	3	6
H. S.	♂	Chromophobe adenoma	25	0	17
J. C.	♂	Chromophobe adenoma	25	10	15
C. C.	♂	Chromophobe adenoma	75‖	2	21
J. J.	♂	Craniopharyngioma	25	6	19
W. R.	♂	Chromophobe adenoma	25	4	30
H. P.	♀	Chromophobe adenoma	25	12	33
M. B.	♂	Glioma, optic chiasm	25	3	37

* ACTH administered intravenously over four hours unless otherwise indicated.
† ACTH given intramuscularly in 25 mgm. doses q6h.
‡ ACTH 25 mgm. given intravenously over four hours on two successive days.
§ This test performed 19 days after test (a).
‖ ACTH 25 mgm. given intravenously over four hours on three successive days.

ever, the definite rise seen in all of these patients was in contrast to the complete lack of response to ACTH found in the patients with primary adrenocortical insufficiency (compare Tables II and III) .

In three patients (W. R., H. P., and M. B.) plasma 17-hydroxycorticosteroid responses to ACTH administration approached those encountered in the normal subjects.

In seven instances, in addition to the determination of plasma 17-hydroxycorticosteroids, urinary 17-ketosteroid and "corticoid" excretion was measured during the 24-hour period which included the four-hour ACTH infusion. Increase in urinary steroid values was found in four cases (Table III, patients J. M., C. C., H. P., and M. B.), while in three patients (R. H., T. R., and W. R.) no increase in urinary values occurred.

D. *Cushing's syndrome*

ACTH tests were performed in three patients with hyperadrenalism due to bilateral adrenal hyperplasia. One patient (S. B.) was studied before surgery, and two (K. R., and C. P.) after the first but prior to a second adrenalectomy.[9] It will be seen in Table IV that the rises in plasma 17-hydroxycorticosteroid levels were in excess of the rises found in the normal subject (compare Table I and Table IV).

In two of the patients it was possible to assess the adrenocortical response to ACTH after bilateral subtotal adrenalectomy. K. R., in whom ACTH caused a rise in plasma 17-hydroxycorticosteroid level to 118 micrograms per cent before the second adrenalectomy, had a postoperative

[9] Adrenalectomies performed by Dr. George F. Cahill.

TABLE IV

Effect of ACTH on plasma 17-OH-corticosteroids of patients with Cushing's syndrome (25 mgm. ACTH administered intravenously over four hours)

Patient	Sex	Lesion	Plasma 17-OH-corticosteroids, *micrograms* %: Before adrenalectomy, Before ACTH	Before adrenalectomy, After ACTH	After adrenalectomy, Before ACTH	After adrenalectomy, After ACTH
S. B.	♀	Bilateral adrenal hyperplasia	34	77	31	61
K. R.*	♀	Bilateral adrenal hyperplasia	20	118	12	30
C. P.*	♂	Bilateral adrenal hyperplasia	28	61	0	8†
L. T.	♀	Adrenal carcinoma	46	62	—	—

* These two patients had had unilateral adrenalectomy prior to ACTH administration. Operation here referred to is the *second* of 2 adrenalectomies.
† This patient had signs and symptoms of adrenal insufficiency following his second adrenalectomy.

TABLE V

Effect of ACTH (25 mgm. intravenously over a four-hour period) on plasma 17-OH-corticosteroids in patients with congenital adrenal hyperplasia

Patient	Sex	Plasma 17-OH-corticosteroids, *micrograms %*	
		Before ACTH	After ACTH
E. B.	♂	7	16
R. W.	♂	5	12
K. A.	♀	17	21
J. L.	♀	9	12
T. K.	♀	0	0
D. P.	♀	20	39

ACTH response of smaller magnitude than that encountered in normal individuals. In C. P., there was clinical evidence of hypoadrenalism after the second adrenalectomy when replacement therapy was withdrawn (*i.e.*, weakness, fever, hypotension, hemoconcentration, and a fall in serum sodium values from 141 to 131 mEq. per L.). At that time, he demonstrated a subnormal rise in plasma 17-hydroxycorticosteroid levels after ACTH (from 0 to 8 micrograms per cent).

A fourth patient with Cushing's syndrome due to adrenal carcinoma was studied (L. T., see Table IV). The control plasma 17-hydroxycorticosteroid level was 46 micrograms per cent, with a rise to 62 micrograms per cent after ACTH.

E. *Congenital adrenal hyperplasia*

Table V summarizes the results of ACTH administration in six patients with congenital adrenal hyperplasia, ranging in age from 6 to 18 years. There appeared to be three patterns of response. In four instances, minimal plasma 17-hydroxycorticosteroid rises occurred after ACTH (see Table V, patients E. B., R. W., K. A., and J. L.). In one patient (T. K.), who exhibited no detectable plasma 17-hydroxycorticosteroid level before ACTH, there was no change. In only one patient (D. P.) was a normal response found.

Similar results have been obtained in other clinics following ACTH administration to patients with this disorder. The observations are summarized in Table VI.

F. *Normal pregnancy*

Three normal women were studied during the third trimester of pregnancy. All three showed rises of plasma 17-hydroxycorticosteroid levels in excess of the rise found in normal subjects. The control values ranged from 32 to 46 micrograms per cent. After intravenous ACTH values ranged from 79 to 111 micrograms per cent.

DISCUSSION

The response of plasma 17, 21-dihydroxy-20-ketosteroids to the intravenous administration of ACTH affords a means of characterizing adrenal function in various disorders of the adrenal cortex. In normal individuals, under the conditions of the standardized ACTH test used in this study, the plasma 17-hydroxycorticosteroid values following ACTH varied from 35 to 54 micrograms per 100 ml. plasma. (These values are above the range found in normal subjects without exogenous ACTH [12].) The plasma levels attained at the end of the ACTH test appeared to be more char-

TABLE VI

Effect of ACTH on plasma 17-OH-corticosteroids in patients with congenital adrenal hyperplasia as reported by various authors

Author	Number of patients	Plasma 17-OH-corticoids *micrograms %*		Route and dose of ACTH administration
		Before ACTH	After ACTH	
Present study	1	20	39	Intravenous, 25 mgm., 4 hr.
	5	0–17	0–21	
Bayliss (14)	1	3	8	Intravenous, 20 mgm., 8 hr.
Bongiovanni (15)	2	3–8	20–28	Intravenous, 0.5 mgm./kgm., 3 to 4 hrs.
	7	1	No significant rise	
Ely et al. (10)	6	2.6	2.6	Intramuscular, 25 to 100 mgm., 2 hr.
Kelley et al. (9)	1	0	11.3	Intramuscular, 25 to 100 mgm.
	2	2.5–7.9	No rise	

acteristic than any set increment over the control plasma levels. This relative constancy of response suggests a certain uniformity in the secretory capacity of the normal human adrenal cortex, under the conditions of this testing method. Similar findings have been reported by Eik-Nes, Sandberg, Nelson, Tyler, and Samuels (11).

Diminished responses to intravenous ACTH were encountered in three groups of subjects: patients with 1) Addison's disease, 2) hypopituitarism, and 3) congenital adrenal virilism due to bilateral adrenal hyperplasia. In the patients with Addison's disease, ACTH consistently failed to cause a rise in plasma 17-hydroxycorticosteroid values. This was the rule whether the period of ACTH administration was brief or prolonged, and whether the control plasma 17-hydroxycorticosteroid level was low or within normal limits. The uniform failure of plasma 17-hydroxycorticosteroid levels to rise following ACTH is at variance with the findings of Eik-Nes and his coworkers (11) who noted appreciable though subnormal plasma 17-hydroxycorticosteroid rises within two hours after the start of ACTH infusions in two of six patients with Addison's disease. In this laboratory, the only patients who as a group have failed to show adrenocortical response to exogenous ACTH have been those with primary adrenal insufficiency.

In contrast to the Addisonian patients, those with hypopituitarism invariably showed an increase in plasma 17-hydroxycorticosteroid values following ACTH. However, in seven of the ten cases studied the magnitude of the increase was much smaller than that found in normal subjects.

In patients with hypopituitarism, it is relatively simple to evaluate thyroid function by means of the basal metabolic rate, the thyroidal uptake of radioactive iodine, the plasma level of protein-bound iodine, and the response of the thyroid to stimulation by thyrotropin. Gonadotropic hypofunction can be documented both by clinical criteria and by the absence of urinary gonadotropins. The assessment of adrenocortical function and of the patient's need for adrenal replacement therapy are not as easily accomplished. The low urinary steroid excretion values and the urinary steroid response to ACTH which have been used as indices of adrenal activity in patients with hypopituitarism, may be influenced by disordered thyroid and gonadal function as well as by the poorly-understood effects of a chronic debilitating disease. At the present time it is not possible to predict with certainty whether an individual patient with hypopituitarism will require cortisone for optimal maintenance. It may be possible when these patients have been followed for a longer period of time to correlate their need for adrenal replacement therapy with the diminished responsiveness to ACTH. Eventually then, the ACTH test may serve as a guide in planning such therapy.

The diminished response of plasma 17-hydroxycorticosteroid levels to ACTH stimulation found in most patients with congenital adrenal hyperplasia is in accord with the results of previous investigations (9, 10, 14, 15). It should be reemphasized that none of the patients in this group had the so-called "salt-losing syndrome," and none had clinical evidence of adrenal insufficiency. The reduced adrenocortical response to ACTH appears to support the hypothesis that patients with this type of adrenal virilism have an inborn error of steroid metabolism characterized by a partial enzymatic block in the synthesis of 17-hydroxycorticosterone from its precursors (15–18).

Two groups of patients showed an exaggerated plasma 17-hydroxycorticosteroid rise after ACTH: 1) patients with Cushing's syndrome due to bilateral adrenal hyperplasia; and 2) normal women in the third trimester of pregnancy. In the patients with Cushing's syndrome, this excessive response occurred even after the removal of one of the hyperplastic adrenal glands. In two of these cases studied following *bilateral* subtotal adrenalectomy, subnormal increases in plasma 17-hydroxycorticosteroids were noted after ACTH. One patient with Cushing's syndrome due to adrenal carcinoma showed a plasma 17-hydroxycorticosteroid rise of much smaller magnitude than that found in the patients with hyperplasia. Other investigators have studied adrenocortical response to ACTH in Cushing's syndrome, using urinary steroid excretion changes as the index of activity. With this method, Laidlaw, Jenkins, Reddy, Harrison, and Thorn (19) found exaggerated responses in patients with bilateral adrenal hyperplasia, and no response in adrenal carcinoma. Kolb, Bruce, Liddle, Miller, and Forsham (20) reported one case of unilateral benign adenoma in

which excessive rises in urinary corticosteroid values were noted following ACTH administration. In Laidlaw's patients with benign adenoma, an excessive rise in urinary corticosteroids was found in one case, while three patients responded normally (19). On the basis of the data now available, the plasma corticosteroid response to ACTH clearly differentiates adrenocortical carcinoma from hyperplasia. Experience with adrenal adenomas has been too limited to permit a definite statement concerning the usefulness of the ACTH test in distinguishing Cushing's syndrome of this etiology from either of the other two categories. In the small number of cases studied postoperatively, the ACTH-corticosteroid response appears to be a useful index of the efficacy of treatment.

During the course of normal pregnancy urinary (21) and plasma (22) corticosteroid levels are known to rise progressively until term. However, the source of these "adrenal-like" steroids has not been clearly established. It is also known that urinary steroid values in patients with Addison's disease show progressive increases with advancing pregnancy (23, 24). Again, the source of these "corticoids" remains a matter of debate. In the one pregnant Addisonian patient studied in this clinic, the rise in urinary steroid excretion values was again observed, and paralleled a rise in plasma 17-hydroxycorticosteroid levels. It seemed important to determine whether the source of these substances could be stimulated by ACTH. Accordingly, a standard ACTH test was performed in the third trimester of the pregnancy. No further increase in plasma 17-hydroxycorticosteroids was demonstrated. This confirms the results of Hills, Venning, Dohan, Webster, and Richardson (24) who noted no increase in urinary "corticoids" after ACTH in two pregnant patients with Addison's disease. These findings prompted studies in normal women in the third trimester of pregnancy. The patients showed increases in plasma 17-hydroxycorticosteroids after ACTH which were far in excess of the normal.

From these data, it seems permissible to draw two conclusions: 1) The increase of Porter-Silber reactive substances, measured as plasma 17-hydroxycorticosteroids which occurred in advancing pregnancy in this patient with Addison's disease, was due to an extra-adrenal source which could not be further stimulated by ACTH;[10] 2) The increasing concentration of these substances in normal pregnancy may be due both to an extra-adrenal source and to the adrenal cortex, but the excessive corticosteroid increase following ACTH must be due to an enhanced responsiveness of the adrenal cortex itself.

SUMMARY

The usefulness of the plasma 17,21-dihydroxy-20-ketosteroid response to intravenously-administered ACTH in the assessment of adrenocortical function has been confirmed. After a standard ACTH test, changes in plasma 17-hydroxycorticosteroid levels were rather constant in normal individuals. Under these conditions, no increase in plasma levels was encountered in patients with Addison's disease. Subnormal rises in plasma 17-hydroxycorticosteroid values were demonstrated in patients with hypopituitarism and congenital adrenal hyperplasia. Adrenocortical responses to ACTH in excess of the normal occurred in women in the third trimester of normal pregnancy and in patients with Cushing's syndrome due to bilateral adrenal hyperplasia. In the latter patients, bilateral subtotal adrenalectomy was followed by subnormal plasma 17-hydroxycorticosteroid response to ACTH.

Addenda

(a) Since these studies were completed Eik-Nes and his co-workers have reported data on the effects of intravenous ACTH administration on plasma 17-OH-corticosteroid levels in various non-endocrine disorders, in Addison's disease, and in one patient with Cushing's syndrome (25).

(b) Two additional patients with Cushing's syndrome due to bilateral adrenocortical hyperplasia have been tested with ACTH in the manner described, and showed a similar hyper-responsiveness.

ACKNOWLEDGMENT

The authors gratefully acknowledge the valuable technical assistance of Miss Elsie Ewen.

[10] The question of whether this source is a maximally-stimulated adrenocortical remnant or an extra-adrenal site (*e.g.*, the placenta) cannot at present be answered. Evidence presented elsewhere suggests that the first alternative is less likely (23, 24).

REFERENCES

1. Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G., Clinical studies with pituitary adrenocorticotropin. J. Clin. Endocrinol., 1948, **8**, 15.
2. Renold, A. E., Jenkins, D., Forsham, P. H., and Thorn, G. W., The use of intravenous ACTH: A study in quantitative adrenocortical stimulation. J. Clin. Endocrinol. & Metab., 1952, **12**, 763.
3. Thorn, G. W., Goetz, F. C., Streeten, D. H. P., Dingman, J. F., and Arons, W. L., Use of the intravenous ACTH test in clinical practice. J. Clin. Endocrinol. & Metab., 1953, **13**, 604.
4. Nelson, D. H., and Samuels, L. T., A method for the determination of 17-hydroxycorticosteroids in blood: 17-hydroxycorticosterone in the peripheral circulation. J. Clin. Endocrinol. & Metab., 1952, **12**, 519.
5. Silber, R. H., and Porter, C. C., The determination of 17, 21-dihydroxy-20-ketosteroids in urine and plasma. J. Biol. Chem., 1954, **210**, 923.
6. Tyler, F. H., Sandberg, A. A., and Eik-Nes, K., Adrenal cortical reserve and its relation to stress. J. Clin. Invest., 1953, **32**, 608.
7. Raile, R. B., Ely, R. S., and Kelley, V. C., Studies of 17-hydroxycorticosteroids in children. II. Response of blood levels to the injection of certain hormones. J. Pediat., 1953, **42**, 46.
8. Bliss, E. L., Nelson, D. H., and Samuels, L. T., Effects of intravenous ACTH on blood levels of 17-hydroxycorticosteroids and circulating leukocytes. J. Clin. Endocrinol. & Metab., 1954, **14**, 423.
9. Kelley, V. C., Ely, R. S., and Raile, R. B., Metabolic studies in patients with congenital adrenal hyperplasia. Effects of cortisone therapy. J. Clin. Endocrinol. & Metab., 1952, **12**, 1140.
10. Ely, R. S., Raile, R. B., Bray, P. F., and Kelley, V. C., Studies of 17-hydroxycorticosteroids. IV. Evaluation of a standard ACTH—17-hydroxycorticosteroid response test in children. Pediatrics, 1954, **13**, 403.
11. Eik-Nes, K., Sandberg, A. A., Nelson, D. H., Tyler, F. H., and Samuels, L. T., Changes in plasma levels of 17-hydroxycorticosteroids during the intravenous administration of ACTH. I. A test of adrenocortical capacity in the human. J. Clin. Invest., 1954, **33**, 1502.
12. Wallace, E. Z., Christy, N. P., and Jailer, J. W., Clinical application of the simplified Silber-Porter method for determining plasma 17-OH-corticosteroids. J. Clin. Endocrinol & Metab., In press.
13. Liddle, G. W., Island, D., Rinfret, A. P., and Forsham, P. H., Factors enhancing the response of the human adrenal to corticotropin: Is there an adrenal growth factor? J. Clin. Endocrinol. & Metab., 1954, **14**, 839.
14. Bayliss, R. I. S., Broadbent, I. E., and Steinbeck, A. W., Congenital adrenal hyperplasia. Effects of corticotrophin and tetrahydrocortisone. Lancet, 1954, **1**, 434.
15. Bongiovanni, A. M., Eberlein, W. R., and Cara, J., Studies on the metabolism of adrenal steroids in the adrenogenital syndrome. J. Clin. Endocrinol. & Metab., 1954, **14**, 409.
16. Jailer, J. W., Virilism. Bull. New York Acad. Med., 1953, **29**, 377.
17. Jailer, J. W., Gold, J. J., and Lieberman, S., 17-α hydroxy-progesterone and 21-desoxyhydrocortisone; their metabolism and possible role in congenital adrenal virilism. In preparation.
18. Dorfman, R. I., The metabolism of adrenal steroids *in* Adrenal Cortex. Transactions of the 5th Conference, 1953, Josiah Macy, Jr., Foundation, New York, 1954, p. 27.
19. Laidlaw, J. C., Jenkins, D., Reddy, W. J., Harrison, J. H., and Thorn, G. W., Diagnosis of hyperadrenocorticism. J. Clin. Endocrinol. & Metab., 1954, **14**, 781.
20. Kolb, F. O., Bruce, J. H., Liddle, G. W., Miller, G. M., and Forsham, P. H., Stimulation by exogenous ACTH administration of an adrenocortical adenoma producing Cushing's syndrome. J. Clin. Endocrinol. & Metab., 1953, **13**, 870.
21. Venning, E. H., Adrenal function in pregnancy. Endocrinol., 1946, **39**, 203.
22. Gemzell, C. A., Blood levels of 17-hydroxycorticosteroids in normal pregnancy. J. Clin. Endocrinol. & Metab., 1953, **13**, 898.
23. Jailer, J. W., and Knowlton, A. I., Simulated adrenocortical activity during pregnancy in an Addisonian patient. J. Clin. Invest., 1950, **29**, 1430.
24. Hills, A. G., Venning, E. H., Dohan, F. C., Webster, G. D., Jr., and Richardson, E. M., Pregnancy and adrenocortical function; endocrine studies of pregnancy occurring in two adrenal-deficient women. J. Clin. Invest., 1954, **33**, 1466.
25. Eik-Nes, K., Sandberg, A. A., Migeon, C. J., Tyler, F. H., and Samuels, L. T., Changes in plasma levels of 17-hydroxycorticosteroids during the intravenous administration of ACTH. II. Response under various clinical conditions. J. Clin. Endocrinol. & Metab., 1955, **15**, 13.

PROCEEDINGS OF THE FORTY-SEVENTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., MAY 2, 1955

PRESIDENTIAL ADDRESS

ACTIVITIES OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

BY ROBERT H. WILLIAMS,* M.D.

The chief objectives of the ASCI are, as stated in the Constitution:

(a) cultivation of clinical research.
(b) unification of science and the practice of medicine.
(c) encouragement of investigation by the practitioner.
(d) diffusion of the scientific spirit among its members.

These are excellent objectives, but the question deserving consideration is, "how well do we attain them?" My impressions are based upon (a) my attendance at all meetings in the last 16 years, except one, during 12 of which I have been a member, (b) many discussions with members and non-members, and (c) correspondence with the members. Recently, I sent a letter to each active member requesting comments on the activities of the Society and inviting opinions particularly in regard to the pattern of the annual scientific program, scientific exhibits and society membership as well as the other roles of the society. I am glad to state that I received approximately 100 replies. I have carefully read and reread all of the letters. It was evident that the members had given much thought to these subjects. The discussions were relatively thorough and contained many very good suggestions. My inquiries were not in the form of a poll, so I am not presenting a statistical survey, but I do wish to indicate certain trends.

Society Membership

The Constitution states, "any physician residing in the United States or Canada who is less than 45 years old, has accomplished meritorious original investigation in the clinical or allied sciences of medicine, and enjoys an unimpeachable moral standing in the medical profession, is eligible to membership." Thirty-eight members said that the present quota for new members is approximately correct, whereas 18 considered it definitely too restrictive. It is evident, however, that most members are familiar with the qualifications of only a few of the nominees. For example, some who endorsed the present quota have, in other letters, said with great conviction that each of their 6 candidates was as good as or better than the average member and certainly should be elected immediately. However, those of us who have studied the qualifications of *all* the nominees know that no more than one-third of their nominees can compete successfully with nominees from other localities. The uninformed state of members causes them to accuse the officers, unjustly, of discrimination, while in reality they are attempting to be very objective. Indeed, an enormous amount of time is spent accumulating the facts relative to the nominees. Many excellent men fail to be elected because the competition is so keen. Year after year some nominees are rejected despite excellent letters from all the active and emeritus members who have written about them. Some of the dissenting letters have come from individuals who have previously held office in the Society. Bitter antagonisms have arisen because the proposers thought that their recommendations had been considered too lightly. The opinion of several members was expressed by one who wrote, "I see no particular purpose served by keeping it (the Society) small unless the smallness of its numbers can really be shown to serve a useful purpose."

My conclusion is that there are at least some years when we should elect more members than we do. As seen in Figure 1, during the last few years the ratio of electees to nominees has decreased significantly. More-

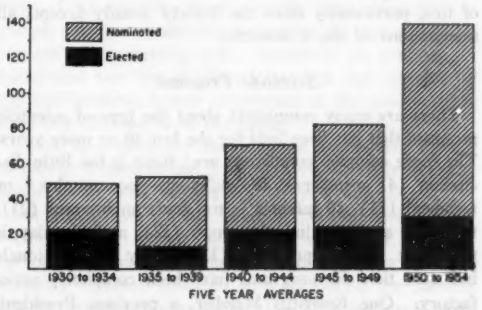

FIG. 1. The decrease, in recent years, in the proportion of nominees elected to membership is immediately apparent. The total number of nominations per year has increased markedly, because of the great increase in clinical investigators. Some members feel that the qualifications of the average nominee in recent years have been superior to those nominated one or two decades ago. In all intervals shown, a greater proportion of nominees are eventually elected than the columns indicate; some nominees are considered for three or more years before election.

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over, it must be emphasized that the number of physicians trained to be clinical investigators is now about 10 times greater than it was 10 years ago. Today, there also is a greater tendency to delay nominations than there was 1 or 2 decades ago. I think the present policy of electing only 35 members (or an equivalent number to those becoming emeritus members if this exceeds 35) is a good standard at present, but I recommend that we change the Constitution to permit election of up to 10 additional members in a given year whenever the Council considers this necessary to prevent the accumulation of a back-log of candidates definitely worthy of election. It would mean more to some individuals to become members at an earlier age.

Since most members of the Society are internists, some think we should have more representatives from the basic medical sciences and other clinical sciences. On the other hand, there are others who think we should have fewer members from the other specialties, stating that specialists in other fields have their respective societies and that some of such members are in our Society to accept the honor and not to contribute to it. My impression is that it is good to have about 15 per cent of the membership composed of other specialists. This experience would enable them to be of still greater assistance to internists in research, in teaching and in other ways.

There are numerous accusations that favoritism is shown to nominees in the Northeastern Coast section, whereas members in this area state that favoritism is shown to other regions. It is my impression that there has not been much of this in recent years, but I think that it would be helpful to increase the number of Councillors to 4 and the number of members on the Nominating Committee to 5. My 3 years' experience on the Nominating Committee emphasized to me the importance of this, particularly since the Society usually accepts all nominations of the Committee.

Scientific Program

There are many complaints about the type of scientific program that has been held for the last 10 or more years. The more common complaints are: there is too little discussion (47 members), the talks are too complex* or technical (21), 10 minutes is too short an interval (21), the talks are not clinical enough (12), and one day is too short for the program (13). Only 29 individuals indicated that the programs have been completely satisfactory. One Emeritus Member, a previous President, wrote me "the program and the Steel Pier are terrible. . . . I do not see why these meetings should be the occasion for carnivals, in which non-members are 10 times as numerous as members." An active member wrote "I will be perfectly honest in stating that in the last few years my interest in the organization has dwindled to rather a low ebb." There has been much dissatisfaction expressed relative to the inadequacy of the discussions. This inadequacy was attributed to the presence of such large crowds, the presiding officer's necessary haste in completing the long program at a fairly reasonable hour, and the failure of the listeners to grasp the methods, techniques, etc., adequate to give a good discussion without becoming embarrassed before such a large audience. In preparing the program this year I wrote one well-qualified person to discuss each paper.

The type of program selected is often criticized, some critics stating that many papers read by title are better than those presented. Frequently, members greatly resent exclusion of their papers from the program. Recently, more than 175 abstracts have been submitted yearly and only 27 selected, although approximately 150 indicate quite significant studies. Having studied the program situation intensively, I believe it can be improved by a change in the pattern. Most members engage in restricted fields of research and have an avid desire to learn the results of others working in the same line. Often, the more details that are given the more they benefit. In papers concerning their own field, there is no difficulty in readily comprehending all the facts. However, intricate discussions of methodology and other minutiae are both uninteresting and incomprehensible to investigators in other specialties. Thus, discouraged and frustrated, they leave the audience. However, for the greatest effectiveness in teaching, research, administration and patient care, it is important that these persons learn of progress in other fields. Some individuals who attend the meeting would be surprised, if subjected to a critical examination upon return home, to find how little useful information they had acquired. On the other hand, there are others who learn a tremendous amount.

With the foregoing considerations in mind, I am recommending that the program be extended to two days with the first day consisting of plenary sessions and the second of four specialty sessions conducted simultaneously.

(A) *Plenary Sessions.* There should be 12 papers in the morning and 10 papers in the afternoon, with a 12-minute limit. It is desirable to have a 15-minute intermission in the morning and in the afternoon. These papers should be of interest to essentially all of the different specialists, and especially to the *clinical* investigators. Presentation of a great deal of complicated data with crowded tables and charts should be avoided. Adequate orientation should be provided for the members who are not engaged in the specialty involved and there should be close clinical applications. Investigations which have a second or third order relationship to clinical problems are better presented in the Specialty Sessions. There probably will be more discussion than in the past, but if not, certain discussers should be invited in advance.

(B) *Specialty Sessions.* Four of these are recommended as a beginning: (1) cardiology, (2) endocrinology, (3) hematology, and (4) others. The last group can be divided as the need develops. Ten-minute presen-

* With the Address a series of 6 slides were shown as a take-off on a very complicated and somewhat irrelevant talk. The slides were overloaded with data, curves, and complex equations and the observations reported had no practical clinical significance.

tations should be adequate. Discussions will unquestionably be much more extensive than in recent years.

Scientific Exhibits

Most of the members indicated approval of the commercial exhibits, particularly those demonstrating new types of apparatus and new techniques. Conversely, the majority stated that exhibits by members of the Society were not appropriate. Since a few exhibits by members are scheduled for this meeting, it will be interesting to ascertain the reaction toward them.

Social Activities

The Society has functioned chiefly as a medium for the presentation of scientific papers. There is relatively little "Society spirit" because the meeting is open to everyone and the members get scattered among the throngs of nonmembers and because no social activities for its membership are sponsored by the Society. The sharp rejection of so many excellent papers and very good nominees has provoked a rather cool reaction from some members: indeed, a few actually have become greatly embittered. Thus, the Society is subject to the same type of criticism as are individual scientists, viz., it contributes greatly to the revelation of scientific facts, but it does little to nurture warm human relationships. One member wrote me ". . . as the crowds at Atlantic City have become so very vast, it has become difficult to meet anyone except by appointment, and often then, only for brief periods of time." Another member states, "I visualize it originally as being a rather small club which had social function and a high scientific standard. . . . it is no longer a social club in any sense of the word. We don't even have an annual banquet, but instead wander over to the Brighton and then down to Hackney's. It is with the greatest difficulty that I am able to remember who is a member and who is not a member." Several other members suggested that the Society have an annual banquet on Monday evening. I am in favor of this for several reasons: (a) the Society is composed of many of the outstanding clinical investigators and it is desirable to have them get together, know one another and experience mutual intellectual stimulation and social pleasure. (b) It is the plan this year for the Council to welcome at a cocktail party on Monday evening, all individuals elected to membership this year and last. It would be good for the entire Society to assume the welcoming role. (c) As discussed in the succeeding section, Monday evening is the best time to hold the Business Meeting. Certain arrangements could be made to permit the banquet to be held at a lower than average cost. Upon completion of the Business Meeting the members could remain in the ballroom for many small social gatherings during the remainder of the evening.

Business Meeting

The Business Meetings in recent years have consisted largely of reports of the Council's actions. The huge audience and the necessary rush to proceed with a full scientific program discourages participation of the Society as a whole in discussion of business matters. Therefore, it is preferable to have the Business Meeting Monday evening, following the banquet. It is anticipated that attendance will be composed almost entirely of active members and that more business matters will be considered than in the past.

As indicated in the list of Objectives of the Society, presented earlier, we are responsible for the *cultivation* of clinical research—not merely for witnessing the harvest, inculcation of science in the practice of medicine, and stimulation of investigation by the practitioner, as well as diffusion of the scientific spirit. The Society has done little to fulfill some of its Objectives. In the replies to my letter to the membership, most of the members indicated that many of them should play a more active role in the formulation of major policies pertaining to teaching and research, but that the problems should be handled by Committees appointed by the Society, or by individual members working with other societies or agencies. Some of the problems confronting full-time clinical teacher-investigators and methods for dealing with them were presented in the Address, but because of lack of space they are to be printed elsewhere in a separate paper.

Summary and Conclusions

Although the American Society for Clinical Investigation is one of the most honored societies in the world, some of its activities need periodic readjustments. At present there are sufficient indications for permitting an increase in membership and a change in the program; I recommend one day of plenary sessions and one day of specialty sessions, better orientation in the presentations and more discussions of the papers.

There is a need for more social intercourse by the members. I recommend a banquet on Monday evening, following the welcoming party for new members.

We should give greater heed to the Objectives of our Society, which include *cultivation* of clinical research. For optimal results attention to many factors is required, including excellent relations with the public, the patients and the practicing physicians. We must be much more active, particularly as individuals, in helping formulate the major policies affecting teaching and research; the detailed discussion of these factors is to be printed subsequently.

ABSTRACTS

1. *Factors Influencing Immunologic Response to Non-Infectious Poliomyelitis Virus Antigens.* JONAS E. SALK,* Pittsburgh, Pa.

The immunologic response to poliomyelitis virus antigen in man is evidenced by two measurable reactions. One is the antibody response itself and the other is the state of hyper-reactivity elicited upon subsequent antigenic stimulation. The hyper-reactive state is characterized by a shorter interval between provocation and secondary antibody response, and also by the very much greater degree of the secondary as compared with the primary response. The degree of this secondary effect is influenced by a number of factors; some of these are: intensity of primary stimulation, intensity of secondary stimulation, and interval between primary and secondary stimulation. The full secondary effect, in man, cannot be elicited unless the secondary stimulation is given several months after the primary. These factors influence the degree to which the serum antibody level tends to persist. Thus, immunologic response in man, following injection of non-infectious poliomyelitis virus vaccine, is affected by previous immunologic experience. The quantitation of experience-induced host factors, and the quantitation of response to different degrees of stimulation, has provided insight into the dynamics of secondary antibody formation and into the dynamics of the immunologic mechanism of defense against paralysis. It now appears that the presence of measurable amounts of circulating antibody may not be the minimal essential prerequisite for immunity to paralysis; but, the minimal requirement seems to be the existence of a hyper-reactive state, of the immunologic mechanism, of such degree that the initiation of viral multiplication after invasion through the natural portal of entry will stimulate promptly the formation of antibody and thereby limit deeper invasion or further multiplication.

2. *Cardiac Lesions in Rabbits After Pharyngeal Infections with Group A Streptococci.* ROBERT J. GLASER,* STEPHEN I. MORSE, JAMES E. DARNELL, JR., and WILBUR A. THOMAS, St. Louis, Mo.

Although the existence of a relationship between group A streptococcal infections and rheumatic fever is generally accepted, the pathogenesis of the latter disease remains unsolved. Recently in this laboratory a method has been devised whereby streptococcal pharyngeal infections can be induced in rabbits, and it has thus become possible to simulate more closely than previously the conditions which obtain in man.

Of eight infected rabbits that constituted the initial experimental group, seven developed striking myocardial lesions, some extending to the endocardium and epicardium. The histopathologic findings which included (a) muscle necrosis; (b) cellular infiltration with histiocytes, lymphocytes, multinucleated giant cells and Anitschkow myocytes; and (c) disruption of collagen and fibrosis closely resembled those of rheumatic carditis in man. Bacteria, viral inclusion bodies and parasites were not demonstrable in the lesions.

In contrast the hearts of four "sham" controls, subjected to identical procedures except that sterile broth rather than streptococcal culture was used as the inoculum, were free of comparable alterations, as were thirty-nine of forty comparative controls. One animal in the latter group had only a single small area of myocardial necrosis.

Because myocardial lesions were observed in two animals after single infections, other experiments were directed toward defining the shortest period of time necessary for the development of changes such as described, and it was found that they occurred in all of five animals sacrificed within seven days after induction of infection.

Thus the production in rabbits of streptococcal infections in the pharynx—the site of predilection of streptococcal infections in man—results in myocardial lesions closely resembling those of human rheumatic fever in almost 100 per cent of animals. This incidence is considerably greater than has been reported in experimental animals by previous investigations using other methods, and attests to the significance of the observations.

3. *Successful Homovital Transplantation in Agammaglobulinemia.* ROBERT A. GOOD and RICHARD L. VARCO, Minneapolis, Minn. (Introduced by Irvine McQuarrie).

During the past year we have discovered and studied eight patients with agammaglobulinemia. These patients have isolated gamma globulin deficiency associated with failure of antibody production. The studies to be reported represent an attempt to interpret the fascinating "experiment of nature" posed by these patients. An associated inadequacy of the hematopoietic reticulum expressed as either neutropenia, lymphopenia, eosinopenia, hyperplasia of the fixed reticulum, and in each patient failure of plasma cell formation in response to antigenic stimulation is described.

In an attempt to elucidate the basis for homotransplantation failure in man, agammaglobulinemic subjects have been provided with homotransplants of skin from unrelated donors. Successful homotransplantation in two children with congenital agammaglobulinemia and prolonged survival of a homotransplant in a patient with acquired agammaglobulinemia resulted. Skin from the agammaglobulinemic patients transplanted to immunologically normal recipients sloughed after 4 to 7 weeks. Rejection of skin homotransplants is associated with the development of local plasmacytosis.

Agammaglobulinemic patients did not have bacterial type hypersensitivity. However, injection of intact white

blood cells from sensitive donors produced this type of reactivity against tuberculin and streptococcal products which lasted at least six months.

Transplantation of lymph nodes from a normal donor to a patient with acquired agammaglobulinemia introduced immunological reactivity, a capacity which was lost when the transplanted lymph nodes sloughed. Acute-phase reactants including C-reactive protein, mucoprotein, fibrinogen, heparin-precipitable protein, erythrocyte sedimentation rates, respond as in normal persons to the stimuli provided by disease and other noxious agents. Observations on gamma globulin concentration, immunological reactivity response of bone marrow made periodically throughout pregnancy on an agammaglobulinemic female and during the neonatal period on her offspring will be described. The implications of these observations on immunological theory will be discussed.

4. *The Relation of Circulating Endogenous Pyrogen to the Cause of Experimental Fever.* ELISHA ATKINS and W. BARRY WOOD, JR.,* St. Louis, Mo.

Although many of the physiologic factors involved in the control of body temperature have been defined, the stimuli which act upon the thermoregulatory centers of the brain to cause the fevers of disease are unknown. The demonstration of a pyrogenic substance extractable from leucocytes has added support to the hypothesis that, in diseases involving inflammation, cell injury results in the release of an endogenous pyrogen which acts as the real cause of the fever. No such pyrogenic substance circulating in the blood during fever, however, has hitherto been identified. Its presence in the blood of rabbits made febrile by the injection of exogenous bacterial pyrogen has been demonstrated in the present study.

By means of a method of passive transfer, the serum of donors sensitized by one or two prior injections, but non-tolerant to typhoid vaccine, was found to contain a rapidly acting pyrogen similar to that obtained by other investigators from leucocytes and their extracts. This pyrogen appeared after clearance of the originally injected vaccine, and its fever-producing activity was unaffected by the inhibitors in tolerant serum. In contrast, the pyrogenic effect of the uncleared vaccine present in serum obtained 5 minutes after injection was markedly inhibited.

These results indicate that a second pyrogen of endogenous origin, apparently arising from damage of host cells, eventually appears in the blood after inoculation of exogenous bacterial pyrogen. The rapid onset of fever following injection of the endogenous factor suggests that it may well be the essential factor which acts upon the hypothalamus to cause fever.

5. *The Enzymatic Formation of Porphobilinogen from δ-Aminolevulinic Acid and its Conversion to Haem.* RUDI SCHMID and DAVID SHEMIN, New York, N. Y. (Introduced by John V. Taggart).

It has previously been demonstrated in this laboratory that δ-aminolevulinic acid is an intermediate in the biosynthesis of porphyrins and of haem, and also that haem synthesis from δ-aminolevulinic acid occurs in a cell-free extract of duck erythrocytes. It has now been possible to isolate by fractional ammonium sulfate precipitation from duck erythrocytes a highly purified protein fraction which converts δ-aminolevulinic acid to the monopyrrole, porphobilinogen.

In an experiment with anaerobic incubation this protein fraction (15 mg. protein-N) with 230 mg. of δ-aminolevulinic acid-5-C^{14} and 30 mg. of glutathione yielded approximately 110 mg. of porphobilinogen; 58 mg. of crystalline porphobilinogen were isolated. The product was identified by elementary analysis, by its conversion to porphyrins on heating in acid, by ascending paper chromatography, and by a positive Ehrlich reaction. The molar radioactivity of the porphobilinogen was found to be twice that of the substrate, demonstrating that 2 moles of δ-aminolevulinic acid combine to form porphobilinogen.

Equal volumes of a cell-free extract of duck erythrocytes were incubated with equimolar amounts of δ-aminolevulinic acid-5-C^{14} (0.018 mc per mM) or with enzymatically synthesized radioactive porphobilinogen (0.036 mc per mM). The hemin samples synthesized from these substrates exhibited the same radioactivity, indicating that porphobilinogen is an obligatory intermediate in the biosynthesis of haem.

Mature human erythrocytes are incapable of forming haem from δ-aminolevulinic acid *in vitro*, but they were found to contain the enzymes capable of converting δ-aminolevulinic acid to porphobilinogen. On the other hand, haem synthesis from δ-aminolevulinic acid occurred in cell-free extracts obtained from erythrocytes of sickle cell anemia, thalassemia major and human cord blood (1.4 per cent reticulocytes, no normoblasts), whereas haem synthesis was insignificant in a case of acquired hemolytic anemia, exhibiting 22 per cent reticulocytes and occasional normoblasts.

6. *Life Span, Glucose Metabolism and Osmotic Fragility of Erythrocytes in Hereditary Spherocytosis.* A. G. MOTULSKY, E. GIBLETT, D. COLEMAN, B. GABRIO, and C. A. FINCH,* Seattle, Wash.

Investigations were performed to define the mechanism of hemolysis in hereditary spherocytosis. *Quantitative aspects:* Erythrocyte destruction rates were increased six to eight times normal. Non-anemic "carriers" were shown to have compensated hemolytic disease. Destruction rates were determined in a patient with an extremely small spleen and in several splenectomized cases. Significantly increased, although compensated, erythrocyte destruction could be demonstrated in three such patients. *Qualitative aspects:* Studies of erythrocyte intermediate glucose metabolism showed a defect characterized by high specific activity of inorganic P 32 phosphate and low specific activity of organic phosphate when P 32 labelled intermediates were separated by paper electrophoresis and chromatography. This metabolic lesion was reversible with glucose and adenosine. Increased osmotic fra-

gility produced by 24 hours' incubation at 37 degrees was partially reversible with these compounds.

Transfusion studies demonstrated that tagged spherocytes from a splenectomized patient became more osmotically fragile when exposed to splenic circulation. Conversely, when spherocytes from an untreated patient were transfused to a splenectomized recipient, their fragility reverted toward normal and approximated that of the recipient's spherocytes. Serial determinations of osmotic resistance by radioactive techniques showed no correlation between cell age and osmotic fragility.

These findings indicate: 1) The bone marrow delivers spherocytes of uniform fragility before and after splenectomy; 2) increased osmotic resistance after splenectomy is related to the absence of splenic stasis; 3) destruction of hereditary spherocytes is unrelated to cell aging and occurs as a random event in the spleen; 4) a reversible metabolic defect associated with glucose metabolism may be demonstrable in the erythrocytes before and after splenectomy; 5) occasionally, the basic defect may be severe enough to produce premature destruction even in the absence of significant splenic tissue.

7. *Sequestration by the Spleen of Red Cells Sensitized with Incomplete Antibody and with Metallo-protein Complexes.* JAMES H. JANDL, Boston, Mass. (Introduced by W. B. Castle).

Studies in patients using $Na_2^{51}CrO_4$-labelled red cells and body surface scanning showed that moderately agglutinated red cells are removed chiefly by the liver, and coarsely agglutinated cells by lung and liver, with subsequent release within minutes of hemoglobin into the plasma. In sharp contrast, globulin-coated but unagglutinated red cells (anti-D sensitized) were rapidly and almost quantitatively sequestered by the spleen, in both D-positive and D-negative subjects; hemoglobinemia did not appear. D-negative red cells treated with anti-D serum survived normally.

Dilute saline solutions of numerous trivalent and bivalent metallic cations caused agglutination of washed normal red cells. Chromic and ferric ions, in the presence either of autologous, homologous, or heterologous sera, or of serum fractions containing globulins, can in addition bind globulins to the red cell surface without producing agglutination. This induces a positive antiglobulin (Coombs) test characterized by a prozone and by agglutination in high dilutions of antiglobulin serum. Red cells so treated were also sequestered preferentially by the spleen, whereas when agglutination was produced by metals in the absence of protein, the lung and liver were the chief sites of destruction.

Red cells sensitized with anti-D serum, or nonimmunologically with a metallo-protein complex, agglutinated when suspended in autologous serum which had been concentrated as little as 1.2 times. Studies on normal dogs, in which splenic blood hematocrits of over 90 per cent were observed, revealed total protein concentrations in the splenic serum of up to 1.5 times that in the arterial serum. This suggests that concentration both of red cells and of plasma proteins is concerned in a physical mechanism resulting in the splenic sequestration of globulin-coated red cells.

8. *The Requirement for the Properdin System in the Hemolysis of Human Erythrocytes Treated with Tannic Acid.* CARL F. HINZ, JR. and LOUIS PILLEMER, Cleveland, O. (Introduced by Thomas Hale Ham).

Normal human group O erythrocytes treated with weak solutions of tannic acid (TA cells) are hemolyzed at 37° C. by normal human serum. The properdin system is required for the hemolysis as evident from the following: 1) Serum from which only properdin has been removed fails to hemolyze TA cells; 2) Addition of properdin to such serum restores the lytic property; 3) Serum from which any component of complement is lacking, serum heated to 52° C., and resin-treated serum are not lytic, and, furthermore, the addition of properdin to such sera does not restore their lytic property. However, the addition of Mg^{++} to resin-treated serum restores lysis. These results indicate that hemolysis of TA cells requires the properdin system.

Previous studies showed that properdin, a recently described serum globulin, is essential to the hemolysis *in vitro* of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH). Thus, TA cells and PNH cells behave similarly in the properdin system, and are the only human erythrocytes so far studied that have a specific requirement for properdin in hemolysis.

Tannic acid is believed to combine with and alter the stroma of the erythrocyte, rendering it susceptible to hemolysis by normal serum. The similarities reported here between the susceptibility of PNH and TA cells to lysis by the properdin system allows further exploration into the basic cellular defect in both the PNH and TA cells.

Work on the possible use of TA cells in the assay for properdin is under way and will be discussed.

9. *The Effect of a Blocking Analogue of Cystine on Leukemia.* AUSTIN S. WEISBERGER* and LEIF G. SUHRLAND, Cleveland, O.

Previous studies indicate that L-cysteine or L-cystine may have an important role in leukocyte metabolism. Cystine deficiency in animals results in leukopenia and decreased incidence of spontaneous or induced leukemia. Cystine deficiency in marrow cultures results in granulocytopenia. Furthermore, leukemic leukocytes exhibit a rapid turnover of L-cystine. These observations suggest that substances inhibiting the incorporation of L-cystine by leukocytes might influence the course of leukemia. Accordingly, the effect of various compounds on the incorporation of radioactive L-cystine by leukemic leukocytes was studied *in vitro*.

Only compounds with certain structural characteristics decreased the uptake of L-cystine. Selenium cystine, in which selenium replaces sulfur in the cystine molecule, was effective in low concentrations in decreasing the incorporation of both radioactive L-cystine and L-methionine.

Other organic and inorganic selenium compounds were ineffective. Selenium cystine decreased the incorporation of S^{35} L-cystine by lymphosarcoma cells in rats. It also inhibited the growth of this tumor whereas other selenium compounds were ineffective.

The effect of selenium cystine was also studied in two patients with chronic myeloid leukemia and in one with acute leukemia. A rapid decrease in leukocyte count and in spleen size occurred in each patient. Initially the daily rate of fall ranged from 25,000 to 50,000 leukocytes per cubic mm. Immature granulocytes disappeared more rapidly than mature granulocytes in the two patients with chronic myeloid leukemia. In the patient with acute leukemia the leukocyte count fell from 505,000 to 25,000 cells per cubic mm. Diphenylselenide, another selenium compound, was ineffective. During therapy the amount of radioactive cystine incorporated by these leukocytes *in vitro* was markedly decreased. Toxic manifestations included nausea, vomiting, anorexia, and alopecia. No hepatic or renal damage was encountered.

These striking effects of a blocking analogue of cystine tend to confirm the importance of cystine in leukocyte metabolism.

10. *On the Mechanism of Secretion of the Sodium-Retaining Hormone (Aldosterone) Within the Body.* JOHN H. LARAGH and HERBERT C. STOERK, New York, N. Y. and Rahway, N. J. (Introduced by Robert F. Loeb).

Aldosterone, a powerful sodium-retaining hormone has been isolated from adrenal glands and chemically characterized. The physiological factors governing its elaboration within the body are not established. Considerable evidence has been presented to indicate that secretion of aldosterone is not under the control of anterior pituitary hormones, and it has been reported that restriction of dietary sodium increases urinary aldosterone.

The authors have previously demonstrated that potassium administration can produce a rise in serum sodium, that sodium depleted subjects may develop sustained hyperkalemia after feeding relatively small amounts of potassium and that hypertrophy of the rat zona glomerulosa can be correlated with level of serum potassium.

The present study was designed to determine effects of changes in sodium and potassium intake and blood concentration on aldosterone secretion. Careful metabolic balance studies were carried out on normal and diabetes-insipidus dogs receiving constant daily food and water. Serum sodium and potassium concentrations were varied over wide ranges and kept abnormal for sustained periods by methods previously reported. Steroid extracts of urine were bioassayed in rats for sodium-retaining property.

Restriction of dietary sodium even when combined with reduction in serum sodium produced only slight to negligible activity of urine extracts unless potassium was provided in the diet. When potassium was given in excess, marked increases to seven-fold biological activity occurred.

The results indicate that potassium is a powerful direct or indirect stimulus to aldosterone secretion. Changes in serum sodium appear excluded as a hormone stimulus. Dietary sodium restriction may exert its effect by modifying serum potassium or Na/K relationships within body water. However, the data best support the hypothesis that change in serum potassium is a regulator, and possibly the primary regulator, of hormone activity.

Similar studies carried out in man will also be reported.

11. *The Metabolic Effects of Metacortandracin and Metacortandralone in Man: A New Series of $\Delta^{1,4}$ Diene Steroids.* MAURICE M. PECHET, Bethesda, Md. (Introduced by Mark Altschule).

A new series of steroids, $\Delta^{1,4}$ dienes, containing two double bonds in ring A of the sterol nucleus were studied in normal subjects and their metabolic effects were compared with those of cortisone and hydrocortisone. Metacortandralone (meticortelone:ΔF), 70 mgm. daily, produced sodium loss as compared with marked sodium retention with hydrocortisone, 150 mgm. daily. The sodium loss with 30 mgm. meticortelone daily was less than with 70 mgm. The endogenous creatinine clearance, which was raised markedly with metacortandracin (meticorten:ΔE) and meticortelone in Addisonian patients, was raised only slightly in the normal subjects. Both meticorten and meticortelone produced the characteristic transitory potassium diuresis on starting and retention on stopping medication as did cortisone and hydrocortisone. Meticortelone is about five times more effective than hydrocortisone in inducing this phenomenon. Seventy mgm. of meticortelone daily produced marked nitrogen loss; this was considerably greater than with 30 mgm. daily. The effect on nitrogen of 30 mgm. daily was comparable to that of 150 mgm. hydrocortisone daily. The eosinopenic effect of meticortelone was five to ten times that of hydrocortisone. These steroids were considerably more active than cortisone and hydrocortisone in producing lymphopenia and leukocytosis. The glucose tolerance curve, normal to begin with, was of a mild diabetic form after the administration of 590 mgm. meticortelone over a nine-day period, but fasting blood sugars remained normal. Inhibition of normal adrenal function occurred with these steroids, as evidenced by diminished 17 ketosteroid excretion. Seventy mgm. meticortelone produced about 50 per cent decrease in 17 ketosteroid excretion and a concomitant six-fold increase in urinary Porter Silber chromogens.

The actions of meticorten and meticortelone are thus qualitatively similar to, albeit quantitatively greater than, cortisone and hydrocortisone, with the notable exception that sodium retention has not been observed with them in man.

12. *The Plasma Protein-Thyroid Hormone Complex in Thyrotoxicosis vs. Euthyroidism in Man.* MILTON W. HAMOLSKY, Boston, Mass. (Introduced by A. Stone Freedberg).

Previous studies from our laboratory have shown that radioactivity of endogenously and *in vitro* labelled thyroid hormone-plasma protein complex of hyperthyroid patients (diffuse toxic goitre) (1) disappeared more rapidly from circulation after intravenous infusion into dogs and (2) was incorporated in greater amounts *in vitro* by rat diaphragm than similarly labelled euthyroid plasma. A method has been developed to study *in vitro* "uptake" of I-131, I-131-1-thyroxine (TX), I-131-1-triiodothyronine (TRI) by human red blood cells from whole blood or blood reconstituted by addition of R.b.c. of 1 donor to plasma or serum from another compatible donor. Under established conditions, euthyroid red cell radioactivity (corrected to 100 hematocrit) was low with I-131 and I-131-1-TX (< 1.0 per cent) but strikingly greater with I-131-1-TRI (13.3 ± 1.9 per cent in 53 runs with bath concentrations of 6 to 149 μgm. $\times 10^{-4}$ per cc.). R.b.c. "uptake" of I-131-1-TRI was consistently greater in hyperthyroid blood. In 54 paired runs at equal radioactivity concentrations, ratios of uptake (hyperthyroid: euthyroid) ranged from 1.3 to 2.7, averaged 1.7 ± 0.3. Uptake was unchanged by (1) prior addition of stable 1-TX or 1-TRI (.006 to 0.26 μgm. per cc. blood) or by (2) separation and reconstitution of R.b.c. and plasma of same donor. However, euthyroid R.b.c. (with or without prior saline washing), showed hyperthyroid uptakes when incubated in hyperthyroid plasma or serum. Conversely, hyperthyroid R.b.c., reconstituted with euthyroid plasma or serum, had euthyroid uptakes. Comparable striking and consistent differences were observed in the two thyroid states under various experimental modifications—(1) pre-heating of plasma before reconstitution, (2) dilution of blood or plasma with saline, (3) admixture of euthyroid and hyperthyroid plasmas in varying proportions. These results are consistent with previous differences observed and indicate (1) a qualitative difference in plasma protein-thyroid hormone complex or (2) plasma factor(s) differentially affecting tissue uptake in the patient with diffuse toxic goitre *vs.* the euthyroid state.

13. *An Abnormality of the Peripheral Metabolism of Thyroxine in Patients with Treated Graves' Disease: The Syndrome of Euthyroidism Associated with Thyroidal Hyperfunction.* SIDNEY H. INGBAR and NORBERT FREINKEL, Boston, Mass. (Introduced by Maxwell Finland).

An abnormally rapid rate of turnover of synthetic radio-1-thyroxine has been found in 5 patients with untreated Graves' disease (half-time, 2.2 to 4.3 days; mean, 3.1). Contrasting values of thyroxine half-time were observed in 11 euthyroid (5.8 to 7.8 days; mean, 6.6), 8 untreated myxedematous (5.9 to 9.0 days; mean, 7.5), 9 treated myxedematous (5.9 to 8.3 days; mean, 6.9), and 3 panhypopituitary patients (8.0 to 9.7 days; mean, 9.0).

In order to ascertain whether this accelerated turnover of thyroxine was the consequence of increased PBI or of the hypermetabolic state, patients were studied during antithyroid and following surgical therapy. In 5 patients, despite clinical and laboratory evidence of restoration of euthyroidism for as long as one year, accelerated degradation of thyroxine persisted (half-time, 3.6 to 4.6 days; mean, 4.2). The data reveal an abnormality of the peripheral degradation of thyroxine which may persist despite amelioration of other stigmata of thyrotoxicity, including elevation of PBI.

Evidence that the rapid turnover of thyroxine observed under these circumstances does not reflect solely nonspecific detoxification or deiodination was obtained in 3 of the latter 5 subjects. In these patients, supranormal thyroidal incorporation of I^{131} was associated with subnormal PBI's (1.2 to 2.8 μg. per cent). Persistently accelerated turnover of thyroxine was sufficient, however, to produce normal values for the total degradation of thyroxine (20.7 to 72.0 μg. iodine per day), which were reflected in clinical euthyroidism (BMR's, -3.5 to $+8.0$ per cent). These findings suggest that at least a large portion of the rapidly degraded thyroxine must undergo true metabolic utilization. An analogous mechanism (*i.e.*, persistently rapid turnover of thyroxine) may contribute to the syndrome of clinical euthyroidism despite augmented thyroidal accumulation of I^{131}, which others have observed in patients with Graves' disease following treatment by surgery or radioactive iodine.

14. *Studies on Indole Metabolism in Patients with Malignant Carcinoid (Argentaffinoma).* ALBERT SJOERDSMA and SIDNEY UDENFRIEND, Bethesda, Md. (Introduced by James A. Shannon).

A syndrome characterized by unusual cutaneous flushing, cyanosis and valvular disease of the right heart has recently been described in association with metastatic carcinoid. Large amounts of 5-hydroxytryptamine (5HTA, serotonin) have been found in carcinoid tumors and in the blood of patients with metastatic carcinoid. Therefore, it has been suggested that the syndrome may be related to the excessive production of 5HTA by the tumor.

5HTA and its metabolic product, 5-hydroxyindole acetic acid (5HIAA), were measured in the blood and urine of normals and in patients with the carcinoid syndrome. Carcinoid patients showed markedly elevated levels and measurement of urinary 5HIAA has served as a simple diagnostic test for the condition.

Detailed metabolic studies were done on a 38-year-old housewife with the typical syndrome. The blood 5HTA level was 4.0 μgm. per ml. (normal 0.2 to 0.4 μgm. per ml.) and the average daily output of 5HIAA was 330 mgm. (normal 3 to 8 mgm.). The concept that tryptophan is a precursor of 5HTA and 5HIAA was confirmed in the human through the administration of C-14 labelled

tryptophan to this patient. Tryptophan loading caused no change in the output of 5HIAA; however, 5HIAA excretion diminished during two days of tryptophan depletion. It is concluded from these studies that the predominant pathway of tryptophan metabolism in this condition is through 5HTA.

This syndrome affords an opportunity to study what is normally a minor pathway of tryptophan metabolism. The idea that 5HTA and tryptophan metabolism might be related to the development of the cardiac lesions is highly speculative but if true would constitute a unique variety of metabolic heart disease. The diversion of tryptophan from normal pathways leading to protein and niacin may be a factor in the nutritional disturbance and in the occurrence of signs of pellagra in some of these patients.

15. *Left Heart Catheterization in Aortic and Mitral Disease.* TRUMAN G. SCHNABEL, JR., WILLIAM S. BLAKEMORE, PETER T. KUO, and STEPHEN B. LANGFELD, Philadelphia, Pa. (Introduced by Calvin F. Kay).

A technique for the simultaneous recording of pressures in the left atrium and left ventricle, or the left ventricle and aorta has been developed. This procedure has been used in nine patients severely ill with aortic or mitral lesions in whom an accurate evaluation of the nature and severity of the valvular lesion was required before recommending a high mortality operation. A number 18 needle was introduced paravertebrally into the left atrium. Two polyvinyl catheters were inserted through the needle. One was left in the atrium, the other was successfully advanced into the left ventricle in six of the patients. Concomitantly a third catheter was inserted percutaneously into the femoral artery and passed into the central aorta. By measuring the pressure gradient across the valve an immediate index of the degree of stenosis or insufficiency is obtained. When combined with estimates of cardiac output, cardiac work, and valvular orifice, size may be estimated, especially in aortic stenosis, with greater accuracy than previously possible. In addition analysis of simultaneous pressure curves provides a means for study of some of the auscultatory findings associated with rheumatic heart disease. In tight mitral stenosis several phenomena were noted: 1) There is a relatively constant pressure gradient between the atrium and ventricle during diastole in patients with auricular fibrillation. This gradient is increased in presystole when normal sinus rhythm exists; 2) the diastolic pressure gradient between the atrium and ventricle persists for an appreciable interval after the onset of ventricular contraction; 3) equalization of atrioventricular pressures occurs late and on the steepest part of the ventricular slope. These findings form in part a basis for interpreting the delay in onset and the loud and snappy character of the first sound in mitral stenosis. One fatality due to hemorrhage occurred in this group of patients.

16. *Influence of Acetyl Strophanthidin on Myocardial Electrolyte Exchange.* HARPER K. HELLEMS, TIMOTHY J. REGAN, and FREDERICK N. TALMERS, Detroit, Mich. (Introduced by Gordon B. Myers).

In vitro studies have suggested that the action of digitalis analogues is principally effected by altered cell membrane permeability to cation. To determine the rate and magnitude of electrolyte changes induced by acetyl strophanthidin, a study was undertaken employing A-V difference of sodium and potassium as a measure of their myocardial exchange after the I.V. infusion of .05 to .10 mgm. per kgm. to 10 anesthetized dogs. Simultaneous blood samples were taken from the femoral artery (A) and catheterized coronary sinus (CS) at approximately 5-minute intervals for periods up to 120 minutes.

Control A-V differences were not significant (K_A 4.05 $\pm$ .16 mEq. per L., K_{CS} 4.01 $\pm$ 1.8 mEq. per L.) (Na_A 150 $\pm$ 1.6 mEq. per L., Na_{CS} 154.2 $\pm$ 2.1 mEq. per L.). After infusion, there was abrupt loss of K from the myocardial cell within one minute, reaching, in 6 minutes, a maximum negative A-V difference of 0.86 $\pm$ 0.15 mEq. per L. (K_A 4.87 $\pm$ 0.19 mEq. per L., K_{CS} 5.73 $\pm$ 0.29 mEq. per L.) ($p < .001$). Myocardial uptake of Na occurred simultaneously with a maximum positive A-V difference of 5.6 $\pm$ 1.4 mEq. per L. ($p < .001$) (Na_A 153.5 $\pm$ 1.86 mEq. per L., Na_{CS} 147.9 $\pm$ 2.72 mEq. per L.). Since the exchange of Na was greater than K and the pH of CS blood decreased, it is probable that H^+ was also released. Significant exchanges occurred with minimal ECG alteration, but the larger exchanges were associated with the more abnormal ECG. After 25 minutes $K_{(A-CS)}$ became positive and $Na_{(A-CS)}$ negative, this phase continuing for 60 minutes. Alterations in cardiac output, respiration, and arterial pH could not account for the electrolyte changes.

As measured by stroke volume, no positive inotropic effect was observed. If this ionic exchange pattern occurs also in the failing heart, it is suggested that altered muscle protein is required for its participation in positive inotropism or that it is not a major factor in this phenomenon.

17. *Dynamics of Ventricular Function in Atrial Fibrillation.* HAROLD T. DODGE and FREDERIC T. KIRKHAM, JR., Bethesda, Md. (Introduced by Robert P. Grant).

The ventricular rate and amplitude of peripheral pulse vary from beat to beat in atrial fibrillation. This is a study of the effects of the beat to beat variations in ventricular filling and aortic pressure on ventricular function in human subjects with atrial fibrillation.

Simultaneously recorded brachial artery pressures and EKGs show that the pulse pressure amplitude may vary widely for a given R-R interval. This is because the amplitude of pulse pressure bears a direct relationship to duration of the preceding R-R interval, but at the same time bears an inverse relationship to pulse pressure amplitude of the preceding beat.

Excursions of the left heart border recorded by electrokymography reflect beat to beat variations in left ventric-

ular end-diastolic and end-systolic volumes. It was found that: (1) there are marked beat to beat variations in both end-systolic volume and end-diastolic volume, (2) end-diastolic volume is a function not only of the duration of ventricular filling, but also of the end-systolic volume from which filling begins, (3) arterial pulse pressure is directly related to end-diastolic volume, (4) beats with larger end-diastolic volumes tend to have smaller end-systolic volumes. These findings account for the inverse relationship of arterial pulse pressure for a given R-R interval to the pulse pressure of the preceding beat.

By using EKY systolic excursions as an index of stroke volume and mean arterial pressure during ejection, a relative index of work per beat has been calculated. "Work" per beat proves to be directly related to the end-diastolic volume. This is the first time "Starling curves" have been plotted for the human heart and provides a new approach to the study of heart function in man.

18. *A Cardiopulmonary Syndrome Associated with Extreme Obesity.* H. O. Sieker, E. H. Estes, Jr., G. A. Kelser, and H. D. McIntosh, Durham, N. C. (Introduced by J. V. Warren).

Four patients have been observed with a combination of extreme obesity, somnolence, Cheyne-Stokes respiration, intermittent cyanosis, polycythemia and rightward electrical axis. Three had signs and symptoms of congestive heart failure. The following data suggest the direct etiological role of obesity in this unusual syndrome.

Respiratory studies were performed on two of these patients, one without heart failure and one after compensation. Both fell asleep easily when undisturbed; and while asleep Cheyne-Stokes respiration, with 15 to 30 seconds of apnea or shallow breathing, was observed. Arterial oxygen saturation continuously cycled from 90 to 95 per cent at onset of apnea to 68 to 74 per cent at onset of breathing, with a corresponding decrease in blood pH of .05 units. Breath-holding produced a 10 to 20 per cent fall in arterial oxygen saturation in 30 to 45 seconds with immediate return after one breath.

The only abnormalities noted in ventilatory function were a 20 per cent decrease in total lung volume and a 50 per cent decrease in expiratory reserve which decreased with recumbency to 150 cc. or 17 per cent of the average normal value. This small functional residual volume may be inadequate to prevent marked swings in arterial oxygenation with shallow breathing.

Right ventricular and pulmonary arterial pressures were moderately elevated, pulmonary capillary pressure was normal. Cardiac output was within normal limits. Ferrokinetic studies indicated a secondary type of polycythemia.

It is postulated that extreme obesity markedly reduces the functional residual capacity. This plus somnolence and decreased sensitivity of the respiratory center leads to periodic hypoventilation and hypoxia, followed by polycythemia, increased pulmonary vascular resistance, and occasionally congestive heart failure. Correction of these abnormalities by weight reduction indicates that this syndrome is reversible. These data implicate marked obesity as the primary factor in this syndrome.

19. *Characteristics of the Human Placenta for Oxygen Transfer.* James Metcalfe, Seymour L. Romney, and Duncan E. Reid, Boston, Mass. (Introduced by C. Sidney Burwell).

A method based upon the nitrous oxide principle has been devised for the estimation of maternal uterine blood flow. This method has been used in the study of a small series of normal humans at term Caesarean section. Uterine blood flow so estimated averaged 500 cc. per minute. The arterio-venous difference for oxygen across the uterus has been determined, which, together with the uterine blood flow, permits calculation of uterine oxygen consumption. The fetal arterio-venous oxygen difference in the umbilical vessels has also been determined in these studies. If the assumption is made that all oxygen consumed by the term uterus is taken up by the fetus, the fetal umbilical blood flow can be estimated.

The oxygen dissociation curve of human infants at birth has been determined, as has the adult oxygen dissociation curve. With these data, plus those derived as described above, certain characteristics of the human placenta with regard to oxygen exchange can be defined. The most interesting of these, from a physiological standpoint, is the mean oxygen pressure gradient across the placenta. This is defined as the average gradient which would have to exist over the total area for gas exchange to satisfy the observations.

Similar data are available in other species. Comparison of the observations in man with those in the rabbit and sheep enables one to compare the efficiency of several placental types in regard to oxygen exchange.

20. *Measurement of Organ Blood Flow Without Blood Sampling.* Hadley L. Conn, Jr., Brookhaven, N. Y., and Philadelphia, Pa. (Introduced by F. D. W. Lukens).

The rapid diffusion equilibrium of inert gases between tissues and blood makes the rate of loss of such gases from tissue dependent upon blood flow when arterial influx of gas is negligible. The use of a radioisotopic inert gas, radioxenon, which is almost entirely cleared from the blood stream with a single passage through the lung, theoretically makes possible organ blood flow determination by external (gamma) counting over the organ in which flow is being measured. The rate of loss of gas thus determined during the period of its exhalation gives blood flow per unit tissue per unit time, when corrected for the tissue-blood gas partition coefficient. No sampling of blood is required. Initially *in vitro* partition coefficients between blood and various dog tissues were determined. Then, with the utilization of an external scintillation counter and collimator, cerebral blood flow was measured in six normal dogs. The results were compared with the Kety-Schmidt method. Muscle and hepatic blood flows were also measured in these dogs,

under normal conditions and after drug administrations, and compared with results obtained by others. The radioxenon and Kety-Schmidt methods for cerebral blood flow agreed well (41 versus 43 ml. per 100 gm. per min.) and the values for muscle and hepatic blood flow were reproducible and within the expected range. However, due to a marked xenon-hemoglobin affinity, large partition corrections were necessary except in the case of the brain in which a high xenon uptake by white matter, nearly twice that by grey, served as a compensating factor. As these corrections were somewhat variable a high order of accuracy for blood flow measurement may not be possible. These findings indicate that further evaluation of inert gas blood flow techniques may be desirable.

21. *A Single Breath Measurement of Pulmonary Diffusing Capacity.* R. E. Forster, C. M. Ogilvie, J. W. Morton, and W. S. Blakemore, Philadelphia, Pa. (Introduced by J. H. Comroe, Jr.).

The modified Krogh single breath test of pulmonary diffusing capacity for CO (D_{CO}) has been simplified for clinical use. The patient, starting from residual volume, has only to make a maximal inspiration of a standard gas mixture containing an innocuous amount of CO (0.3 per cent) and 10 per cent helium, hold this breath for 10 seconds and breathe out; a sample of alveolar gas is analyzed for CO (infra red meter) and for helium (catharometer). The test (1) requires less than two minutes of the patient's time, (2) requires no blood samples, (3) can be repeated in several minutes, (4) has a reproducibility of approximately 10 per cent, (5) is not invalidated by uneven ventilation, since the simultaneous inspiration of helium and CO permits correction for this, and (6) can be performed at rest or on exercise.

D_{CO} measured by this method in resting normal subjects varied from 15 to 45 ml. per min. per mm. Hg (positively correlated with body surface area). It increases markedly during and for two minutes after exercise and varies less than 10 per cent with changes in posture and lung volume. The test is being used for the routine measurement of diffusing capacity in patients with cardio pulmonary disease and has proved to be useful in identifying those patients with impaired function. None of 20 patients with a variety of severe pulmonary diseases experienced any difficulty in performing the test although many of them had vital capacities of less than 1300 ml. Abnormal values as low as 6 ml. per min. per mm. Hg have been encountered. The method has also been applied to individual lungs, using bronchial catheterization.

22. *A Comparative Study of Intrapulmonary Gas Mixing and Functional Residual Capacity in Pulmonary Emphysema, Using Helium and Nitrogen as the Test Gases.* John Hickam,* and Regina Frayser, Durham, N. C.

Mixing of inspired gas throughout the lungs is somewhat uneven in normal persons and may be markedly uneven in patients with lung disease. A measure of intrapulmonary gas mixing can be derived from following the rate at which a test gas is washed from the lungs of a subject who is first equilibrated with the gas and then breathes a mixture which does not contain it. From the latter part of the washout time-concentration curve it is possible to calculate the volume and ventilation rate of a space (the "slow space") which, if homogeneously ventilated, would produce the same excretion curve. Using helium as the test gas, very large slowly ventilated spaces are often found in cases of lung disease, particularly emphysema. In addition, the functional residual capacity (FRC) can be measured from the amount of test gas washed out of the lung in a given time plus that which is estimated to remain in the slow space.

In 10 patients with moderate to severe emphysema comparative measurements of gas mixing and FRC have been made using both nitrogen and helium as test gases. Nitrogen was measured with a nitrogen meter and helium with a katharometer. FRC measurements agreed well. For the group as a whole, nitrogen and helium slow spaces were not significantly different in size or ventilation rate. In a few subjects nitrogen spaces were consistently larger and slower than helium spaces. As demonstrated by studies on a model, this effect can be caused by a combination of the greater diffusibility of helium and a tendency of the slowest space to rebreathe into a somewhat faster space.

In general, intrapulmonary mixing measurements using nitrogen and helium agree well. Occasional, consistent differences may be useful in understanding some of the mechanics of intrapulmonary gas mixing.

23. *Clinical and Biochemical Changes in Patients with Phenylketonuria on Restricted Phenylalanine Intake.* Frank H. Tyler,* James F. Bosma, and Marvin D. Armstong, Salt Lake City, Utah.

In the hereditary disorder, phenylketonuria, mental retardation is associated with an anomaly which interferes with the normal oxidation of phenylalanine. As a result of this enzymatic deficiency, the plasma phenylalanine level is abnormally high on a normal dietary intake, and a variety of abnormal metabolites appear in the plasma and urine. Clinically, convulsions, spasticity and dermatitis are common manifestations in addition to the mental deficiency.

During the past three years, five phenylketonuric children have been fed synthetic diets free of phenylalanine. Institution of this diet resulted in prompt lowering of serum phenylalanine and disappearance of the abnormal metabolites. By the return of small amounts of normal dietary protein to the diet, approximately normal levels of serum phenylalanine were maintained for periods of three to twelve months.

All of the known biochemical abnormalities have been reversed consistently. The dermatitis has disappeared. The other clinical effects have occurred less rapidly and are more difficult to interpret. Convulsive disorders have

ceased without anticonvulsant medication. Electroencephalographic abnormalities have diminished progressively. Improvement in mental and motor performance, although variable in degree, has been observed in each child. In general, the younger the patient, the shorter the period and the less the degree of retardation, the better has been the magnitude of improvement noted. In two infants, addition of phenylalanine to the diet has resulted in clinical and biochemical relapse. Subsequent depletion has restored the previously noted improvement.

Our observations suggest that some toxic effect results from the prolonged high phenylalanine level or the biochemical abnormalities which this induces. This impairs mental and neurologic functioning in a fashion which is only partially reversible. It should be emphasized that it is impossible to determine at present whether or not prolonged phenylalanine restriction will permit normal intellectual development in certain of these patients.

24. *Effects of Dietary Fats on the Serum Lipides of Human Subjects.* E. H. AHRENS, JR.,* T. T. TSALTAS, JULES HIRSCH, and WM. INSULL, JR., New York, N. Y.

Previous clinical studies have demonstrated that isocaloric substitution of plant fat for animal fat in the diet causes a prompt and striking decrease in the concentrations of cholesterol and phospholipides in the serum. To explore this phenomenon, current experiments have tested the effects of feeding single well-defined fats of plant or animal origin to three normocholesteremic and four hypercholesteremic patients in successive five-week periods. Use of orally fed formulas as the sole source of nutrients (Am. J. Clin. Nutr., 2: 336, 1954) assured precision in control of all intakes. The basic formula consisted of fat, protein, and carbohydrate in caloric proportions of 40–15–45 per cent. Body weights were maintained constant throughout the 20 to 50 weeks of each patient's study. Measurements of free and total cholesterol, phospholipides, and triglycerides were made weekly.

The lowest serum lipide concentrations occurred during intake of corn oil formula. These levels were lower than those obtained during ingestion of olive, lard, or coconut oil formulas, or during the feeding of an isocaloric solid diet with similar P-F-C composition and with all fat of plant origin. Incremental additions of cholesterol to the corn oil formula caused no elevation of serum cholesterol until very large amounts (4 gm. per day) were given. When the proportion of calories fed as corn oil was varied from 40 to 70 to 10 per cent, holding protein and caloric intakes constant, one patient showed a cholesterol rise on 10 per cent and prompt decline on 70 per cent intake, while in a second patient lipide levels were unaffected.

Conclusion: The reduction of lipide levels following the feeding of plant fats is dependent upon the specific fat fed, is not due to the decreased intake of cholesterol *per se*, and may be more pronounced with a high intake of fat.

25. *Inhibition of HCl Formation in the Human Stomach by Diamox®; The Role of Carbonic Anhydrase in Gastric Secretion.* HENRY D. JANOWITZ, DAVID A. DREILING, and FRANKLIN HOLLANDER, New York, N. Y. (Introduced by Louis J. Soffer).

The effect of large doses of the carbonic anhydrase inhibitor Diamox® on histamine-stimulated gastric secretion was studied in 15 human subjects and on basal unstimulated secretion in 10 subjects, before and after the intravenous administration of the sodium salt in the dose range 32 to 144 mg. per kg. body weight, given over the course of one hour. Disturbing side-effects were not elicited with doses of 100 mg. per kg. or less. Significant reversible inhibition of acid secretion occurred when doses of 73 mg. per kg. or higher were used. The reduction in HCl output following histamine stimulation ranged from 67 per cent to 100 per cent (mean = 82 per cent) of control values; a similar reduction of basal secretion was also demonstrated. The 100 per cent inhibition is in contrast to a maximum of 97 per cent in the pouch dog; this difference is ascribed to intragastric neutralization. Previous animal studies have shown that the patterns of secretion of sodium and potassium in gastric juice are unaffected by this compound. Studies based on the topical application of this compound to the mucosa of canine pouches have indicated that these effects are due to a local action on the carbonic anhydrase of the stomach, and not necessarily related to circulating plasma levels of drug. They are also independent of alterations in the plasma electrolytes. The present evidence is in keeping with the hypothesis that carbonic anhydrase activity during acid secretion serves to neutralize alkali formed within the parietal cell, simultaneously with HCl, and furnishes bicarbonate as the major (but not sole) anion for exchange with chloride of the plasma. Without this enzyme activity the rate of formation of HCl is markedly depressed.

26. *The Constant Relationship Between Extracellular and Intracellular Buffering with Varying Degrees of Metabolic Acidosis.* WILLIAM B. SCHWARTZ,* KARL J. ØRNING, and RICHARD PORTER, Boston, Mass.

It is known that with large mineral acid loads a significant fraction of retained hydrogen ions is buffered in sites other than extracellular fluid or blood, but the severity of intracellular acidosis with varying degrees of extracellular acidosis is unknown. To explore this relationship, distribution of hydrogen ions among body buffers was calculated during intravenous hydrochloric acid administration in 21 intact unanesthetized dogs.

In 13 experiments HCl was administered continuously at approximately 80 microequivalents per Kg. per min. An initial large fall in extracellular [HCO_3] was followed by progressively smaller reductions. Calculated hydrogen ion distribution and loss of cellular potassium suggested that in mild acidosis extracellular and blood buffers are utilized first and that intracellular buffering occurs chiefly with severe acidosis.

Because of the possibility that this pattern might have resulted from delayed extracellular mixing or slow

transcellular cation exchanges, eight additional experiments were performed in which equilibrium was allowed to occur between short successive acid infusions. [HCO_3] fell sharply during acid administration but more than 50 per cent of this deficit was restored in two hours of equilibration. Comparable [HCO_3] depression occurred with each acid infusion regardless of the absolute bicarbonate level. A curve defining successive bicarbonate levels at equilibrium was roughly linear, and the calculated contributions of extra- and intracellular buffers with progressive acid increments was approximately constant. Intracellular potassium loss was roughly constant in all but the first period, in contrast to the apparent late shift during continuous acid infusions. Urinary ammonium and titratable acid increments were relatively negligible.

The data indicate that the apparent, large, early extracellular and blood buffer utilization in continuous acid infusions is an artefact. Actually, extracellular and intracellular buffering have a fairly constant relationship at all degrees of metabolic acidosis. Serum [HCO_3] appears to reflect linearly whole body buffer stores in acidosis produced by mineral acids.

27. The Effect of Respiratory Alterations of pH on the Internal Equilibrium of Potassium. BELDING H. SCRIBNER and JAMES M. BURNELL, Seattle, Wash. (Introduced by Robert S. Evans).

Studies of acid-base disorders have not separated changes in the internal equilibrium of potassium from changes in total body potassium. In fact, the observation that muscle potassium is low in chronic metabolic alkalosis has led to the assumption that, even in the absence of renal regulation of body potassium, alkalosis causes transfer of potassium out of cells and acidosis causes transfer into cells. We are presenting evidence that the transfer actually occurs in the opposite direction.

Plasma potassium levels were measured during alterations in blood pH in dogs with ligated ureters under Pentothal® anesthesia. Respiratory acidosis (pH 7.0) was induced in five dogs by inhalation of 30 per cent CO_2-70 per cent O_2, and the mean plasma potassium level rose from 4.2 mEq. per L. to 7.5 mEq. per L. in four hours, eight times that of control dogs. Respiratory alkalosis (pH 7.8) was induced in five dogs by mechanical hyperventilation and the mean plasma potassium level fell from 4.5 mEq. per L. to 2.9 mEq. per L. in four hours. Altered equilibrium was maintained for several hours and only after restoration of normal pH did the plasma potassium gradually return to control levels.

These data suggest that intracellular buffering involves transfer of potassium and when pH changes are abrupt buffering may take several hours to reach equilibrium.

We present the hypothesis that acidosis lowers and alkalosis raises the intracellular-extracellular potassium concentration ratio. This alteration of the ratio persists as long as the pH change persists. The low muscle potassium in chronic alkalosis must be due solely to increased renal excretion of potassium.

The influence of pH on the internal equilibrium of potassium must be considered when interpreting the serum potassium level of patients with acid-base imbalance.

ABSTRACTS TO BE READ BY TITLE

Arterial Unsaturation, Venous Admixture, and Porto-Pulmonary Anastomoses in Patients with Cirrhosis of the Liver. WALTER H. ABELMANN, PAUL CALABRESI, GERTRUDE KRAMER, WILLIAM F. MCNEELY, and MICHAEL A. GRAVALLESE, JR., Boston, Mass. (Introduced by Laurence B. Ellis).

Arterial blood of patients with hepatic cirrhosis without ascites may be unsaturated for oxygen. The mean saturation of 25 patients was 92.6 per cent (S.D. 2.1 per cent). In 19 of these the arterial oxygen partial pressure was measured (mean 74 mm. Hg; S.D. 10 mm. Hg) and found significantly decreased (normal mean 92 mm. Hg; S.D. 8 mm. Hg). Resting alveolar-arterial oxygen pressure gradients at two levels of inspired oxygen were determined in 9 patients. All room air gradients (mean 27 mm. Hg) were above normal limits. Gradients became normal (mean 8 mm. Hg) during low oxygen breathing. In 5 of these patients the state at the two levels of inspired oxygen appeared sufficiently comparable to permit quantitation of distribution and diffusion factors according to Riley and Cournand. These 5 patients showed normal lung volumes, ventilation, and diffusion capacity for oxygen. Dead space ventilation was normal in 1 and elevated in 4 (28 per cent to 42 per cent). Venous admixture was increased (range 8 per cent to 20 per cent; mean 13 per cent). These findings suggest an abnormal ventilation-perfusion relationship; they do not differentiate between perfusion of underventilated alveoli and true venous admixture.

Post-mortem portal venograms performed in 9 cirrhotics, after ligation of the venae cavae, suggested that portopulmonary anastomoses might function as a channel for such venous admixture. In all 9 cases the injection mass filled paraesophageal veins, which were dilated and tortuous, and extended upward beyond the level of the carina. The paraesophageal veins anastomosed freely with mediastinal veins, and through these with pericardial and pleural veins. These venous plexi were in communication with the azygous and hemiazygous veins. Occasionally, anastomoses could be seen between mediastinal veins and peribronchial veins at the tracheal bifurcation. In 2 cases the injection mass was found in the pulmonary veins, in the left auricle, and in the left ventricle, while the pulmonary artery and aorta were free of mass.

The Osmotic Adjustment of the Erythrocyte in States of Altered Serum Sodium Concentration. JAMES W. AGNA, and HARVEY C. KNOWLES, JR., Cincinnati, O. (Introduced by Richard W. Vilter).

Preliminary studies have shown a relation between the concentrations of serum sodium and erythrocyte potassium per whole cell. This relation was considered a result of

osmotic adjustment of the erythrocyte to alterations of extracellular fluid tonicity. Investigations were carried out to substantiate this relation and to elucidate the mechanism of erythrocyte osmotic adjustment.

Studies were made on 10 normal subjects; 11 patients with adaption hyponatremia (absent or unsatisfactory response to salt therapy); 6 patients with depletion hyponatremia (beneficial response to salt therapy); and 6 patients with hypernatremia. Patients were classified by serum sodium concentration regardless of other electrolyte derangement.

The relations of the concentrations per liter of water of serum sodium to erythrocyte potassium and to erythrocyte potassium plus sodium were linear and highly significant. In adaption hyponatremia, expressed per solids, there was a gain of cell water with no change in potassium or sodium content. In depletion hyponatremia there was both a gain of cell water and a decrease of potassium and sodium. In hypernatremia the cells gained potassium, probably lost water, and showed no change in sodium content. In no instance was abnormally high sodium content found. No correlation was noted between the erythrocyte concentration and the clinically estimated total body content of potassium.

The findings suggest that osmotic activity of the erythrocyte occurs *in vivo* in a manner similar to that found *in vitro*, and that osmotic adjustment occurs by both water and electrolyte shifts.

Effect of Potassium on Intracellular Bicarbonate of the Renal Cortex. HELEN M. ANDERSON and GILBERT H. MUDGE,* New York City, N. Y.

Extracellular alkalosis of potassium depletion is associated with increased bicarbonate reabsorption and aciduria. Cooke and others have shown that this condition is characterized by an intracellular acidosis of skeletal muscle. It has been previously proposed that the stimulus for the elaboration of an acid urine might be an intracellular acidosis of the renal tubules. Reported efforts to demonstrate this in intact rats have been unsuccessful.

Tissue slices from rabbit kidney cortex lost K^+ and gained Na^+ when prepared in isotonic NaCl. They were incubated in Warburg flasks in buffered bicarbonate with substrate. Paired slices were incubated with and without the addition of K^+ (5 to 10 mEq. per L.) to the external medium; total osmolarity was kept constant by adjusting NaCl. The gas phase was 5 per cent CO_2 to 95 per cent O_2. After incubation tissues were analyzed for acid labile bicarbonate (Conway) and other electrolytes.

Final tissue concentrations were: (without K^+ added) —K^+, 32; HCO_3^-, 12.5; (with K^+ added)—K^+, 71; HCO_3^-, 16.6 (all values mM per kg. wet wt.). These differences are statistically highly significant. With K^+, the higher tissue HCO_3^- was associated with a correspondingly lower tissue chloride. The sum of Na^+ and K^+ did not vary significantly. Similar changes were noted when chloride of the medium was replaced by nitrate.

Assuming the free diffusion of CO_2, the results indicate an intracellular acidosis in K^+ depleted tissues. The effect of added K^+ is due to its cellular accumulation since no changes in tissue bicarbonate were observed when K^+ uptake was inhibited by dinitrophenol or anaerobic incubation.

Intracellular acidosis has been produced by K^+ depletion of kidney slices in an *in vitro* system. Direct extrapolation to the intact animal is complicated by the possibility that the tubular cells may not be homogeneous in terms of this phenomenon.

Forearm Metabolism in Man. Inadequacy of Carbohydrate Oxidation to Account for Resting Oxygen Consumption. REUBIN ANDRES, GORDON CADER, and KENNETH L. ZIERLER,* Baltimore, Md.

A dominant concept concerning skeletal muscle is that it depends for its energy upon carbohydrate consumption. This concept has been tested and found wanting by the following experiment.

Blood flow to the forearm (hand occluded) and arteriovenous concentration differences (brachial artery-deep antecubital vein) were measured. The product of these values is the rate of removal of a metabolite from or of its addition to forearm blood, largely by skeletal muscle. In 11 subjects at rest and after approximately 16 hours' fasting, lactate production accounted for about one-third the glucose removal. In the steady state, glucose removal minus lactate production defines the rate of oxidation of glucose. Under the above conditions, glucose oxidation accounted for only a minor fraction of forearm oxygen consumption (mean, 23 per cent; median, 16 per cent).

This appraisal depends upon the assumption that during the period of study—16 hours after the last meal—carbohydrate stores in muscle were not consumed. Even if this assumption be false, it is possible to estimate the magnitude of carbohydrate storage and assess its role as substrate. To this end heavy oral glucose loading was imposed. Glucose uptake then transiently exceeded immediate oxidative substrate requirements of the forearm. This stored excess carbohydrate, if oxidized exhaustively, could account for continuing oxygen consumption for only a few hours.

It is concluded that oxygen consumption in human skeletal muscle at rest serves mainly the metabolism of non-carbohydrate substrates. That these substrates are chiefly lipid is suggested by the respiratory quotient of forearm blood which was 0.77 ± 0.04 (S.E.M.) in the 11 subjects.

Some Biologic Properties of Doubly Encapsulated Pneumococci. ROBERT AUSTRIAN* and HARRIET P. BERNHEIMER, New York, N. Y.

From pneumococcal transformation reactions, doubly encapsulated organisms forming simultaneously polysaccharides types I and III or types III and V have been isolated. These cellular types result from the interaction of desoxyribonucleates from pneumococci of type I or type V with the cells of a pneumococcus derived from

capsular type III. In each instance, the introduction of the genetic unit for the production of a new capsular polysaccharide into cells of the strain derived from type III is followed by a significant increase in the production of type III polysaccharide as well as by the formation of polysaccharide of a different serologic type.

Cells of doubly encapsulated variants give a positive quellung reaction with type specific antiserum against each of their capsular components. Exposure to the soil bacillus enzyme which specifically hydrolyzes type III polysaccharide results in loss by the cell of the positive quellung reaction with type III antiserum, but reactivity with the antiserum to the second capsular polysaccharide is retained. When doubly encapsulated strains are grown in the presence of antiserum against either capsular component, the cells are agglutinated. In mice, both varieties of doubly encapsulated pneumococci are highly virulent. Mice can be protected against a hundred lethal infecting doses of such organisms, however, by antiserum against either of their capsular components. Such protection is not afforded by a single antiserum when infection is induced with mixtures of two pneumococcal strains each producing but one of the capsular polysaccharides of the doubly encapsulated variant.

The experiments provide another basis for explaining serologic cross reactions in pneumococcus and demonstrate that antigen-antibody reactions involving either of two surface components of the cell may result in protection against lethal infection.

Demonstration of Humoral Factor (or Factors) in Experimental Hypersplenism by the Lactating Rat Technique. MARIO BALDINI, Boston, Mass. (Introduced by William Dameshek).

Splenomegaly and the "macromolecular syndrome" (Huepner) were induced in rats by the injection of methyl cellulose intraperitoneally. The rats developed pancytopenia in association with a hyperplastic bone marrow, *i.e.*, possible "hypersplenism." Pancytopenia did not develop in previously splenectomized rats. In "hypersplenic" rats, splenectomy resulted in normalization of the blood picture.

Whether the pancytopenia of "hypersplenism" is due to increased destruction of the various cellular components or to humoral factors resulting in marrow inhibition is not clear. The question of a humoral factor was studied with the lactating rat technique.

Rats born of "hypersplenic" mothers but lactated by normal lactating females beginning with the first 48 hours of life showed normal body weights and blood pictures.

Rats born of normal mothers but fed from "hypersplenic" mothers soon after birth, showed a slower body growth and developed anemia after 13 days of lactation. The leukocyte values varied greatly, while thrombocytopenia appeared more frequently. The reticulocytes were slightly increased.

Normal baby rats fed from "hypersplenic" mothers splenectomized soon after parturition did not develop anemia. Rats fed by lactating female rats with anemia due to repeated bleeding showed no abnormality of growth or anemia.

The organs of the anemic baby rats showed no distinct pathologic changes and no methyl cellulose could be demonstrated in the reticulo-endothelial cells throughout the body.

Conclusion: The anemia induced in baby rats fed by "hypersplenic" lactating females is due to the presence of a humoral factor residing in the enlarged, pathologic spleen of the mothers. The nature of the humoral factor has not been elucidated.

Acute Effects of Mercurial Diuretics on the Normal Human Kidney. EARL S. BARKER and JOHN KAPP CLARK,* Philadelphia, Pa.

Renal hemodynamics, oxygen consumption, electrolyte excretion and tubular function were measured in 26 normal men by clearance techniques (usually including renal vein catheterization) before and for approximately two hours after 2 cc. of meralluride (Mercuhydrin®) or mersalyl (Salyrgan®) intravenously.

Decreased PAH transport (E_{PAH} or Tm_{PAH}) was evident within 10 minutes, maximal by 30 to 45 minutes and, with mersalyl, appeared recovering late in the study. Depression of E_{PAH} averaged 4.5 per cent after meralluride and 22.2 per cent after mersalyl. Tm_{PAH} was depressed to 74 per cent of control values by meralluride and to 21 per cent by mersalyl.

Excess urinary chloride lost during the entire period of observation was 132 mEq. with mersalyl and 58 mEq. with meralluride. With both agents, rates of chloride excretion were greatest at the end of the experiment (long after maximal effects on PAH transport). Sodium excretion approximately paralleled chloride; potassium tended to decrease and magnesium showed a definite increase.

While these differences between the two agents are highly significant, they may indicate a more intense but briefer action for mersalyl rather than greater total activity.

Although rates of diuresis varied considerably (variable water load), progressively increasing hematocrits indicated dehydrating effect definitely greater with mersalyl, but not correlating well within the group with chloride or PAH effects.

Post-mercurial values for glomerular filtration, renal blood flow and oxygen consumption did not change significantly with either drug. While considerable decreases suggesting real drug effects occurred in a number of subjects, they did not correlate with any of the effects discussed above.

These data indicate specific mercurial blocking effect not dependent on general metabolic depression, and further suggest that effects on PAH and electrolytes involve different systems as evidenced by their magnitude and separation in time.

Acquisition of Staphylococci by Hospitalized Patients. CARL A. BERNTSEN, JR., New York, N. Y. (Introduced by Ralph Tompsett).

Several previous reports have described the replacement of drug sensitive by drug resistant strains of staphylococci in hospitalized patients. The present report describes the new acquisition of staphylococcus aureus by hospitalized patients originally free of staphylococci and demonstrates the differences in this phenomenon in the treated and untreated patients.

At Bellevue Hospital from September 1944 to March 1955, daily nose and throat cultures have been obtained from 212 patients in a general medical ward. On admission 31 per cent were carriers of staphylococcus aureus. The present report concerns the remaining 147 patients who were free of staphylococcus aureus on admission. During hospitalization and treatment of one group of 75 patients, thirty-nine or 52 per cent acquired staphylococcus aureus. Of these twenty-seven or 36 per cent of the total became consistent carriers. In the remaining 72 untreated patients, sixteen or 22 per cent acquired staphylococcus and 11 per cent became consistent carriers.

Coincident with increase in carriers of staphylococcus in the treated group there occurred a second change serving to distinguish treated from untreated groups. In the treated group initially there were twenty-three patients or 31 per cent with beta hemolytic streptococci. After treatment only six patients or 8 per cent carried beta streptococci. The majority of these again acquired beta streptococci more than a week after their treatment was stopped. In contrast 61 per cent of untreated patients carried the beta streptococcus on admission and 44 per cent retained it on discharge. The accelerated fall in beta streptococcus carrier rate in the treated group likely is a consequence of the use of tetracycline. Possibly the increase in carrier rate of staphylococcus aureus in the same group depends upon decrease in beta streptococci for there appear no other changes in nose and throat flora.

These findings suggest that both treated or untreated patients free of staphylococci on admission become carriers during hospitalization. However, the frequency of acquisition of staphylococci in the treated group is more than three times that of untreated patients.

The Control of the Circulating Leukocyte Level. H. R. BIERMAN,* K. KELLY, S. COBLENTZ, F. CORDES, and D. LEFF, Duarte, Calif.

The constancy of the leukocyte count in the peripheral blood of normal subjects is remarkable. Despite marked deviations above or below normal range due to physiological or pharmacological stimuli, the normal level is, after test agents are given intravenously, regained promptly—usually within 4 to 10 minutes.

In an effort to study production and delivery of leukocytes into the blood, together with the constancy of this inviolate level, large numbers of leukocytes were withdrawn from dogs on eight occasions by employing the Cohn blood fractionator. One and two-tenths to 6.4 billion leukocytes (12 to 36 per cent of the total circulating number) had to be removed before a significant drop in count was observed. At least 6.4 billion leukocytes had to be removed to produce a leukocyte count below 5,000 per cu. mm. The leukocytic response to epinephrine was employed to denote the availability of cells in the tissue reservoir. To obtain valid data concerning production of leukocytes, the tissue reservoir had to be depleted. At this point, there was no leukocyte response to epinephrine challenge. The return of leukocytes toward normal from the leukopenic state occurred within 2 hours approaching 160 million leukocytes delivered per minute. The bone marrow exhibited both prompt and delayed evidence of proliferation.

During the period of leukocyte withdrawal, the plasma globulins were reduced. The reappearance of the beta globulins preceded that of the alpha and gamma globulins on each occasion.

It would appear that there is a reservoir of leukocytes in the tissues divided roughly into two components—a readily available (RA) and a non-readily available portion (NRA). The NRA compartment appears to be in equilibrium with the RA component and the latter appears to be related to the peripheral circulating leukocyte level. The peripheral WBC level appears to be maintained as long as cells are available in the RA reservoir. A depleted RA reservoir is the major stimulus to marrow production.

The significance of the findings in terms of leukocyte production, delivery and homeostasis will be presented.

Immediate Changes in Renal Function during Sympatho-Adrenal Stimulation and Blockade as Estimated by a One-Minute Clearance Period Technique. WILLIAM D. BLAKE,* and AGNAR A. STRAUMFJORD, Portland, Ore.

The error inherent in calculating glomerular filtration (GFR) during a changing urine flow (V) is caused by delay in change of urine concentration due to tubular transit time. In dogs about 85 per cent of tubules have a delay of approximately one minute. (Calculated from data of Chinard.) Hence one-minute-clearance periods will give reasonable estimates of GFR if, for each value of V, the concentration obtained in the following period is used in the calculation. Studies were done on anesthetized dogs with urine collected by catheters inserted up to the renal pelves bilaterally. During "control" periods V was maintained at more than 1½ ml. per min. V was altered by administration of epinephrine or norepinephrine either intravenously or by catheter directly into the renal artery or by splanchnic stimulation or blockade. When either hormone was infused into the renal artery, there was parallel decrease in V, total solute excretion and GFR with little or no change in urinary concentration of creatinine (U_{Cr}). Concentration of sodium in urine (U_{Na}) rose only transiently whether or not sodium was the chief urinary solute.

Whenever sympatho-adrenal activity was altered by other means (direct or indirect splanchnic stimulation), there were always reciprocal changes in V and U_{Cr} with little or no change in GFR. U_{Na} changed in a direction opposite to that of V only when sodium was the chief urinary solute. During glucose diuresis V and U_{Na} changed in the same direction. Under both conditions rates of water and sodium excretion changed in the same direction. It is concluded that, during sympatho-adrenal activity, acute changes in V and sodium excretion are partially independent of change in GFR. Further, sympatho-adrenal activity is not simulated by infusion of either epinephrine or nor-epinephrine directly into the renal artery.

Changes in Body Composition and Exchanges of Water During the Course of Acute Renal Failure. L. W. Bluemle, Jr., H. P. Potter, and J. R. Elkinton,* Philadelphia, Pa.

Eight adult female patients with acute renal failure were studied by balance technique. Changes in total body water (ΔW) and total body fat (ΔFat) were calculated from changes in body weight, balance of solids and the metabolic mixture; changes in total body fat-free solids (ΔFFS) by difference. Data are presented as mean values with standard deviations per 24 hours per standard 70 kg. body weight for the following three phases: oliguria (average 5 days studied), early diuresis (average 9 days), and late diuresis (average 7 days).

Changes in body composition for the three phases, respectively, were as follows (grams): ΔW -13 ± 323, -479 ± 257, -58 ± 154; ΔFat -206 ± 38, -175 ± 103, -123 ± 81; ΔFFS -32 ± 18, -71 ± 33, -85 ± 44. The corresponding rates of water exchange were as follows (ml.): exogenous water intake 1083 ± 455, 3097 ± 790, 3933 ± 825; water of oxidation (H_2O_{ox}) 319 ± 37, 358 ± 111, 324 ± 75; endogenous preformed water released by tissue catabolism (H_2O_{pf}) 130 ± 61, 145 ± 59, 120 ± 52; insensible water loss (IL) 1134 ± 138, 1275 ± 393, 1168 ± 270; sensible water loss (urine, vomitus, etc.) 313 ± 123, 2732 ± 835, 3398 ± 698.

The results indicate a decrease in total body fat and fat-free solids in all phases, the latter decrement increasing with respect to the former during the course of diuresis. The greater loss of total body water during early diuresis reflects the excretion of water accumulated from exogenous and endogenous sources in excess of the usual ratio to fat-free solids. Exogenous water required in excess of sensible loss to maintain total body water in proportion to total fat-free solids ($IL - [H_2O_{ox} + H_2O_{pf}]$) was 685 ± 133, 771 ± 307, 724 ± 215 ml. per day.

The Effects of Sodium Chloride on the Biophysical Characteristics of Sodium Hyaluronate. B. Blumberg, G. Oster, and K. Meyer, New York, N. Y. (Introduced by C. Ragan).

In a previous paper, the workers reported on light scattering studies on the size and shape of the hyaluronate particle. The data suggested that the particle is a "swollen" meshwork or syncytium of the linear polymer, free draining and easily deformable, whose outline is a sphere or near sphere. Hyaluronic acid is an important constituent of the ground substance. It has been suggested that it is of importance in support, storage, ion transport repair, and other vital physiological functions. It was felt that changes in the physical nature of the hyaluronate solutions might have a profound effect on these functions and we have, therefore, studied the effect of the sodium ion on biophysical measurements of hyaluronate solutions.

Protein-free hyaluronates of analytically high purity prepared by an alcohol precipitation method from human umbilical cord and streptococcal capsule were studied. Measurements of flow birefringence, viscosity, sedimentation and light scattering were made on solutions of hyaluronate buffered with pH 5.0 acetate containing salt at various concentrations. With increase in salt concentration, flow birefringence decreased. Intrinsic viscosity, sedimentation and the amount of light scattered, all extrapolated to infinite dilutions, decreased. Moreover, the major changes in these measurements occurred with the addition of small amounts of salt to the solution, with little or no change with the addition of salt beyond a sodium ion strength of approximately .13.

Interpretation of these data in terms of physical models can be only approximate and general. However, the data extrapolated to infinite dilution suggest that the particles aggregate in a low salt concentration environment and disaggregate as salt is added. Regardless of the interpretation of the extrapolated values of the data, it is clear that solutions of hyaluronate undergo marked physical changes when salt concentration is lowered and that the material is particularly sensitive to such changes in a region of physiological interest. These physical changes may be of importance in the role of the ground substance in salt and water metabolism, edema, inflammation, and transport exchange mechanisms.

Investigation of Tolerance to Bacterial Endotoxin with Radiochromium Labelled E. Coli *Endotoxin.* A. I. Braude, Francis J. Carey, and Margaret Zalesky, Dallas, Texas. (Introduced by Donald W. Seldin).

The nature of tolerance to endotoxin was examined by correlating the fate of injected radioactive endotoxin with degree of susceptibility to its action. The endotoxin was extracted from *Escherichia coli* and then firmly labelled by incubation with $Na_2Cr^{51}O_4$. Radioactivity proved directly proportional to toxicity.

Distribution of radioactivity was determined after injection of lethal doses of labelled endotoxin into the circulation of mice and rabbits subjected earlier to the following manipulations with non-labelled *E. coli* endotoxin or X: irradiation:

1. No manipulation.
2. Repeated injections to produce tolerance. The LD_{50} for mice was markedly elevated and the fever index of rabbits greatly reduced.

3. X: irradiation to increase susceptibility of mice.

4. Repeated injections followed by injection-free intervals to permit loss of tolerance. Circulating precipitin titers for endotoxin remained high.

5. X: irradiation before repeated injections of mice to produce moderate tolerance without circulating antibody.

Susceptibility was directly related to the speed with which endotoxin disappeared from the blood. In normals, circulating endotoxin disappeared within 2 hours and large amounts appeared in the liver. With increased resistance, 95 per cent disappeared from the circulation within 15 minutes and passed chiefly into lung and liver. In irradiated animals with increased susceptibility, by contrast, the circulating level never dropped below 50 per cent.

When induced tolerance lapsed but antibodies persisted, circulating endotoxin rapidly disappeared into the lung; after 2 hours, however, pulmonary concentrations fell almost to zero and circulating endotoxin reappeared in high concentrations. Irradiated tolerant animals, lacking precipitins, manifested little immediate pulmonary removal of endotoxin but within 30 minutes circulating concentrations fell permanently below 10 per cent.

These results indicate that tolerance depends on speedy and permanent removal of circulating endotoxin into hepatic and other cells. Although not essential for tolerance, circulating antibody may contribute to it by inducing rapid but temporary pulmonary removal which by itself cannot provide protection.

The Hydraulics of Overriding the Aorta. P. Brostoff, S. Rodbard, and J. Margolis, Chicago, Ill. (Introduced by Louis Katz).

The principles governing flow when the aorta overrides the right ventricle were studied by means of an integrated circulation model. When right ventricular pressure was less than aortic diastolic pressure, there was the expected left to right shunt across the interventricular defect but no shunt from right ventricle directly into the overriding aorta. By creating pulmonic stenosis the right ventricular pressure was increased so that for a period it was higher than aortic diastolic but less than left ventricular pressure. Under these circumstances a bidirectional shunt appeared. The right ventricular to overriding aorta shunt began as soon as right ventricular pressure exceeded aortic pressure and continued until the pressure in the aorta exceeded that in the right ventricle. This shunt took place during early systole. When left ventricular pressure exceeded right ventricular pressure a left to right shunt across the ventricular septal defect began and this occurred later than the right to left shunt. There was shunting from right ventricle directly into the overriding aorta and across the ventricular septal defect into the left ventricle during the period when right ventricular pressure was greater than aortic diastolic pressure and left ventricular pressure. As right ventricular pressure continued to increase the shunted fluid travelled preferentially from right ventricle directly into the aorta. Since model and animal experiments have demonstrated only unidirectional shunting in pure ventricular septal defect, it is concluded that overriding of the aorta is necessary for the production of bidirectional shunting.

Metabolism of the Conjugated 17-Hydroxycorticosteroids. Harold Brown and Edwin Englert, Jr., Salt Lake City, Utah. (Introduced by B. V. Jager).

Inasmuch as a considerable portion of the plasma 17-hydroxycorticosteroids (17-OH-CS) is conjugated, a study of this fraction in various clinical states should contribute to the understanding of corticosteroid metabolism.

After a standard one-half hour infusion of hydrocortisone in normal subjects, the levels of the conjugated 17-OH-CS in the plasma reached a peak in two hours and then declined more slowly than the levels of the free 17-OH-CS. In patients with cirrhosis, the peak plasma levels of the conjugated 17-OH-CS were considerably lower and the levels of the free 17-OH-CS declined more slowly than in normal subjects. In patients with uremia the plasma levels of conjugated 17-OH-CS prior to infusion were elevated. After the infusion there was a very high and sustained level of the conjugated 17-OH-CS, although the level of the free material in the plasma declined at an almost normal rate.

After standard infusions of tetrahydrocortisone (tetrahydro E), dihydrocortisone (dihydro E) and tetrahydrohydrocortisone (tetrahydro F) into normal subjects, the initial plasma levels of conjugated 17-OH-CS were at a peak which was much higher than that achieved with comparable hydrocortisone infusions.

In the patients with cirrhosis, tetrahydro E infusions produced the same pattern of conjugated 17-OH-CS levels in the plasma as in the normal subjects. After the infusion of dihydro E or tetrahydro F, however, there was a delay in the attainment of peak levels.

These studies suggest that: (1) the metabolism of hydrocortisone involves a process of reduction and subsequent conjugation; (2) in cirrhosis of the liver, there is an impairment of reduction but not of conjugation; (3) hydrocortisone is converted ultimately to tetrahydro E which is conjugated and excreted *via* the kidney; (4) in renal failure there is an accumulation of this conjugated 17-OH-CS in the plasma.

The Effects of Surgical Procedures on Simultaneously Determined Radiosodium, Radiosulphate, and Radiopotassium Spaces in Human Subjects. Belton A. Burrows,* Donald J. Davis, John F. Kelly, Anthony A. G. Lewis, and Joseph F. Ross,* Boston, Mass.

The apparent volumes of distribution, or spaces, of radiosodium, radiosulphate, and radiopotassium have been compared in human subjects before and after elective surgical procedures which did not produce gross changes in fluid and electrolyte balance. Following the simultaneous intravenous administration of radiosodium and

radiosulphate the rate of decline in plasma activity, expressed as per cent of dose corrected for renal excretion, is similar for the two substances during the first one to two hours. The radiosodium and radiosulphate spaces calculated from the plasma activity at twenty minutes correspond closely to the spaces derived from the extrapolation of subsequent plasma activities back to zero time, and are of equivalent value as an index of extracellular fluid volume.

The twenty-minute radiosodium space is approximately three-fourths the twenty-four-hour value, suggesting that one-fourth of "total exchangeable" sodium is non-extracellular and yet metabolically active. For radiopotassium the initial rate of decline in plasma activity is similar to that for radiosodium. After this period of mixing, presumably with extracellular fluid, the plasma activity of radiopotassium falls more rapidly than that of radiosodium and requires a longer period of equilibration to approach relatively constant values, at approximately twenty-four hours.

The results indicate that the response to surgical trauma does not influence the apparent rates of equilibration of radiosodium, radiosulphate, or radiopotassium; the determination of "total exchangeable" sodium and potassium; or the partition of exchangeable sodium between extracellular and non-extracellular moieties, using the twenty-minute radiosulphate or radiosodium space as an index of extracellular fluid volume. In the patients studied before and one week following elective surgical procedures there was no consistent change in these measurements.

Ventilation, Oxygen Tension and Acid-Base Adjustments During Recovery from Congestive Heart Failure. DOUGLAS CARROLL,* Baltimore, Md.

Serial studies of arterial blood oxygen and carbon dioxide tensions and body weight were made in four patients recovering from combined right and left heart failure. In three it was possible to obtain repeated ventilation and oxygen consumption determinations. During the severe edematous stage of heart failure, carbon dioxide and oxygen tensions were low, whereas ventilation and oxygen consumption were high. With cardiac compensation, there was a gradual rise in carbon dioxide and oxygen tensions, although oxygen tensions tended to remain low normal. Ventilation and oxygen consumption fell, but ventilation never returned to normal values, even with optimal cardiac compensation.

The studies indicate that during severe combined right and left ventricular failure, there are severe abnormalities in the distribution of gas to the lungs. These changes are believed to be caused by extravasation of fluid around the pulmonary capillaries and edema of the walls of bronchioles or smaller air passages.

An increased oxygen consumption at rest during the severe stages of heart failure gradually returned to normal as cardiac compensation occurred. This high oxygen intake was thought to be expended in the performance of the increased work of breathing necessitated by the excessive turgidity of the lungs associated with heart disease.

The hyperventilation present when optimal cardiac compensation had occurred suggests that some pulmonary congestion exists despite lack of signs or symptoms.

Finally, the return of ventilation to its baseline (albeit high) prior to complete loss of body weight supports the view that the fluid shifts which occur in the lesser circulation are relatively small and occur before greater circulation fluid shifts have been completed.

Further Studies on the Abnormalities in the Metabolism of Copper in Wilson's Disease. G. E. CARTWRIGHT,* J. A. BUSH, H. MARKOWITZ, J. P. MAHONEY, and C. J. GUBLER, Salt Lake City, Utah.

The ceruloplasmin level, measured by an immunologic technique, was 34 (27 to 38) mg. per 100 ml. in 10 normal subjects and 9 (2 to 19) mg. per 100 ml. in 14 patients with Wilson's disease. No correlation was observed between the ceruloplasmin level and the duration or severity of the clinical manifestations of the disease. Analyses of tissues of one control subject and one patient with Wilson's disease revealed small quantities of ceruloplasmin in the liver and kidney but none in spleen, brain, bile, or erythrocytes.

Three normal subjects, following the oral administration of Cu^{64}, excreted 66, 83, and 95 per cent of the activity, respectively, in the stools and less than 0.1 per cent in the urine. Three patients with Wilson's disease excreted 35, 57, and 82 per cent in the stools and 2, 5, and 2 per cent, respectively, in the urine.

Following the intravenous administration of Cu^{64} to three normal subjects, 11, 14, and 16 per cent of the activity was recovered in the stools and 0.3 per cent in the urine. Three patients with Wilson's disease excreted 2, 2, and 3 per cent in the stools and 4, 5, and 8 per cent in the urine. The activity in the plasma of the normal subjects decreased rapidly over the first 4 hours and then increased during the period up to 48 hours after the injection. The activity in the initial phase was not precipitable with anti-serum to ceruloplasmin, whereas that present in the plasma at 48 hours was almost completely precipitated. In the patients with Wilson's disease no secondary increase in plasma activity took place. The activity present in the plasma at 48 hours was almost entirely in the supernatant solution after precipitation of the ceruloplasmin.

The significance of these observations will be discussed.

Association of Goiter and Hypothyroidism. C. E. CASSIDY and E. B. ASTWOOD,* Boston, Mass.

If simple goiter is an expression of impaired thyroxine synthesis hypothyroidism should sometimes coexist. However, the association has seldom been recorded except in chronic thyroiditis, extreme iodine deficiency, o from known thyroid-inhibiting substances. Observatio of goiter and myxedema attributed to excess iodine Lipiodol (Raben, J. Clin. Endocrinol. & Metab.,

469, 1953) in one case and from KI in asthma remedy in another (VanderLaan—in preparation) prompted a survey of patients with simple goiter seen in the past two years. Of about 150 cases of non-toxic goiter, 5 were judged on clinical grounds to be hypothyroid and 4 fully myxedematous. The ages were 26 to 50; 3 were men. Estimated thyroid size ranged from 30 to 80 gm. Twenty-four hours I^{131} uptake was normal in 4 patients (14, 28, 40 and 45 per cent) and low in 4 (2.2, 3.9, 4.8, and 7 per cent). Protein-bound iodine concentrations were 0.6 to 4.0 (mean 2.3) μg. per cent. Medication with thyroid relieved the symptoms and signs in all, and caused regression of the goiter in 5 of 8 patients. In no instance were there local or systemic manifestations of an inflammatory process, or known exposure to antithyroid substances or unusual diets. The cause of the disturbance was obscure and the observation that some patients remained well when thyroid medication was stopped suggested that the etiologic factor was transitory.

The Effects of Intravenous Sodium Glutamate on Blood "Ammonia," Keto-Acids and Amino Acids in Patients with Hepatic Coma. THOMAS C. CHALMERS, FRANK L. IBER, HYMAN ROSEN, and STANLEY M. LEVENSON,* Washington, D. C.

Sodium glutamate is occasionally effective in alleviating the coma of severe hepatic disease. In an effort to elucidate the mechanisms involved, blood "ammonia," alpha-keto glutarate, pyruvate, and citrate were measured in 7 patients who eventually died in coma, and in 3 of the patients, one of whom awoke temporarily, individual amino acids were determined.

A possible rationale for glutamate therapy was demonstrated in one patient with rapidly progressing coma. "Ammonia," pyruvate, and 15 of 17 plasma amino acids rose to more than triple their pre-coma, near-normal values, but glutamate, aspartate, and alpha-keto-glutarate decreased.

Six patients in coma from 8 to 96 hours received glutamate 9 times (24 grams over 3 to 4 hours). "Ammonia" decreased on 8 occasions (mean decrease 94 μ-moles per liter, S. E. 37) and α-keto-glutarate increased 8 times (mean 36, S. E. 17). There were no consistent changes in pyruvate and citrate.

In a patient whose coma was unaffected by glutamate, alpha-amino-nitrogen rose from 2,840 to 5,470 μ-moles per liter (4.0 to 7.6 mg. per cent). Aspartate, alanine, and the amides (glutamine and asparagine) accounted for 87 per cent of the increase in amino acids, excluding glutamate which rose from 152 to 957 μ-moles per liter. The blood "ammonia" dropped from 140 to 88 micromoles per liter, alpha-keto-glutarate and pyruvate rose from 30 to 140 and 155 to 260, respectively.

In the only patient who awoke following the infusion "ammonia" dropped from 148 to 102, and α-keto-glutarate rose from 53 to 76, pyruvate dropped from 222 to 106 and α-amino nitrogen rose from 563 to 4,761 μ-moles per liter (0.8 to 6.7 mg. per cent). Aspartate and alanine accounted for 63 per cent of the increase in amino acids, excluding glutamate (17 to 3,730). In this patient the amides decreased.

It is concluded that although a drop in ammonia and rise in α-keto-glutarate following glutamate infusion suggest a beneficial effect, these changes can occur without any sign of improvement. In contrast to previous supposition the drop in ammonia is not always accompanied by a rise in amides.

The Distribution of "Nephron Delay Time" in Normal Man. A. W. CHILDS, H. O. WHEELER, B. COMINSKY, E. LEIFER, O. L. WADE, and S. E. BRADLEY,* New York, N. Y.

The dimensional differences between nephrons suggests that there is a comparable functional variation. Evidence of such a functional non-uniformity was adduced in a recent study (Trans. Assoc. Am. Phys., 65: 147, 1952) of the time required for filtrate to flow from the glomerulus to the bladder (nephron delay time). Analysis of the disparities between the amounts of inulin filtered and the amounts appearing in the urine during successive ten-minute periods following rapid intravenous injection indicated that 60 per cent of the filtrate reached the bladder in the first ten minutes, 30 per cent required twenty minutes, and 10 per cent required thirty minutes or longer.

These findings indicated a considerable range in functional capacity of the nephrons that seemed consistent with other evidence. Since the analysis requires graphic integration of the relationship between changing concentrations of inulin in plasma and urine, the derived values depart from accuracy in the extent to which finite intervals of time are substituted for infinitesimals. Because ten-minute periods might thus have introduced a serious error, nephron delay was determined in ten human subjects on the basis of two-minute collection periods. A much more restricted range of variation was detected.

On the average, 1 per cent of the filtrate required two minutes to reach the bladder, 21 per cent required four minutes, 62 per cent required six minutes, 14 per cent required eight minutes, and 1 per cent required ten minutes. In four patients with severe renal disease, mean nephron delay was prolonged and the frequency distribution widened. It is probable that still shorter periods of collection would further reduce the apparent variation. Within such a narrow range, laminar flow could be an important determinant of the pattern of delay.

Renal Excretion Patterns of Some Electrolytes and Non-Electrolytes. FRANCIS P. CHINARD* and THEODORE ENNS, Baltimore, Md.

The renal excretion patterns of various substances following a single circulation through the kidneys have been determined in dogs. The test substances, together with a glomerular substance (inulin or creatinine), are injected instantaneously into one renal artery. Urine is collected from each ureter separately for 30 periods each of 30 seconds' duration. Correction for recirculation is made by period by period subtraction of the amounts excreted by the control kidney from the amounts excreted

by the kidney into which injection was made. The fractions of the injected substances excreted in each period, corrected for recirculation, are plotted against time to give the excretion patterns; appearance, modal, and mean transit times are calculated from these data. There are no major differences in the excretion patterns or transit times of simultaneously injected glomerular substances. Appearance and transit times of Na-22 and Cl-36 are approximately equal and less by about 30 seconds than the corresponding times for creatinine. Half the eventually excreted Na-22 (and Cl-36) is in the urine when 25 per cent or less of the eventually excreted creatinine is in the urine. Substantial fractions of the urinary sodium and chloride ions appear to bypass a portion(s) of the tubular segments. The transit times of *p*-aminohippurate are greater by about 45 seconds than the corresponding times for creatinine. This delay and the rapid uptake of PAH from blood suggest that the slow process in PAH secretion is the transport across the tubular cells. Excretion patterns of K-42, and of labelled water, glucose, and urea will also be presented.

A Method for the Coupling of Protein Antigen to Erythrocytes: Use of the Method in the Diagnosis of Tuberculosis. LEON R. COLE, J. JAY MATLOFF, and VIRGINIA R. FARRELL, Boston, Mass. (Introduced by Kendall Emerson, Jr.).

A technique has been devised whereby protein antigen is coupled to formalized erythrocytes, and such antigen-coated erythrocytes used to measure antibody. This method provides a stable antigen preparation.

Human erythrocytes are treated with formalin by the method of Flick (1948). After removal of the formalin, clumps are dispersed by mixing in a Waring blender for 20 minutes. The formalized cells are then treated with tetrazotized benzidine. Tuberculin PPD, added at this point, couples onto these treated cells. The PPD-coated erythrocytes are suspended in 0.5 per cent normal guinea pig serum (N.G.P.S.). Twofold dilutions of test sera, from 1:5 to 1:640, are prepared in 1.5 per cent N.G.P.S. To each tube is added a drop of PPD-coated cells. Agglutination patterns are read after standing overnight at 3°C. The sensitized, formalized erythrocytes are stable for well over a week, but require rehomogenizing before each use. The presence of PPD on the formalized cells has been demonstrated by bioassay. Tetrazotized benzidine couples proteins mainly through linkages with their tyrosine and histidine groups. The present technique thus might be applicable to the study of antibody response to a variety of agents, including certain bacterial proteins, antibiotics and hormones, some of which have been demonstrated or suspected to have antigenic properties.

Eighty-four patients with active pulmonary tuberculosis were studied. In most the tuberculous activity was of recent onset. Serum titers of from 1:40 to 1:640 were observed in 76 per cent. Eighty-six hospital patients with other illnesses served as controls. In the control series, 67.5 per cent of patients had titers of $< 1:5$, and 90 per cent fell into a group with titers of 1:10 or less; 8 per cent were 1:20, and 2 per cent were 1:40. Certain correlations between the clinical status of the tuberculous and nontuberculous patients and their titers will be discussed.

The Distribution of Circulating Blood Within the Splanchnic Vasculature. B. COMINSKY, J. R. K. PREEDY, R. HAYS, and H. O. WHEELER, New York, N. Y. (Introduced by Franklin M. Hanger).

Changes in the size of the liver and spleen observed during circulatory adjustments suggest that the splanchnic vasculature may serve as a "reservoir" from which blood may be discharged on need. Measurements of "circulating splanchnic plasma volume" in intact dog and man by a regional dilution technique (Trans. Assoc. Am. Phys., **46**: 294, 1953) also indicate that marked changes in volume may occur. This technique does not appear to measure blood sequestered within the splenic pulp nor does it yield information regarding the behavior of the various components of the splanchnic vasculature during changes in total volume. A means of evaluating the distribution of circulating blood within the splanchnic bed based upon the measurement of "splanchnic hematocrit" is reported in this paper.

The circulating splanchnic plasma volume (SPV) was measured by the regional dilution technique, using I^{131} labelled human serum albumin and circulating splanchnic red cell volume (SRCV), using P^{32} tagged red cells in 11 intact and 8 splenectomized dogs, anesthetized with Nembutal ®. The splanchnic hematocrit was calculated by dividing the value for splanchnic red cell volume by the value of the sum of splanchnic plasma and red cell volumes (SRCV/SPV + SRCV). This figure was always less than peripheral arterial hematocrit, averaging $79.4 \pm (\sigma)$ 8.9 per cent of peripheral hematocrit in intact animals and 71.8 ± 18 per cent in splenectomized dogs, a statistically insignificant difference. This finding indicates that the circulating splanchnic blood volume is for the most part confined to very small blood vessels, presumably the hepatic sinusoids. Allen and Reeve (Am. J. Physiol., **175**: 218, 1953) have shown that the hematocrit of pooled hepatic sinusoidal blood is 69 per cent of arterial hematocrit. On the basis of this figure it may be shown that approximately 77 per cent of the circulating splanchnic blood volume lies within the sinusoids.

Measurements of the Mechanics of Respiration in Newborn Infants. C. D. COOK, J. M. SUTHERLAND, S. SEGAL, R. B. CHERRY, M. B. MCILROY, J. MEAD, and C. A. SMITH, Boston, Mass. (Introduced by C. A. Janeway).

Simultaneous and continuous recordings of respiratory volume and intraesophageal pressure (as a measure of intrapleural pressure change) were obtained 39 times in 23 normal newborn infants ranging in weight from 2.4 to 3.8 Kg. and in age from 1 hour to 7 days. Lung compliance and resistance as well as the work of respiration were calculated from these data. Five similar studies

in two infants severely ill with neonatal respiratory distress (the hyaline membrane syndrome) were carried out.

These data indicate that the mean compliance of the normal newborn infant's lung is approximately 5 ml. per cm. H_2O (range 2 to 9) and the mean resistance approximately .03 cm. H_2O per ml. per sec. (range .005 to .13). The work of respiration for an average resting 2.5 Kg. newborn infant is found to be 1600 gm. cm. per minute with approximately 70 per cent of the work done against elastic resistance and 30 per cent against viscous resistance. In contrast to these data, the observations on the two infants with respiratory distress show a marked reduction in compliance (1 and 0.6 ml. per cm. H_2O, respectively). There is also a marked increase in the work of respiration, a change primarily related to differences in elasticity and to the increased respiratory rate.

The application of a simplified formula (Work = 0.6 PV̇) for the calculation of the minute work of respiration proposed by one of the authors (M. B. McI.) is discussed in relation to the present data. The present data on the mechanics of respiration in normal newborn infants will be correlated with similar data for adults.

The Volume of Distribution of High Molecular Weight Dextran and Its Relation to Plasma Volume in Man. ALBERT B. CRAIG, JR. and CHRISTINE WATERHOUSE,* Rochester, N. Y.

The fractionation of dextran has permitted studies with a polysaccharide which has molecular weights much greater than most serum proteins. Intravascular retention of dextran is responsible for its usefulness as a plasma expander. Theoretically, if such a substance were given in small quantities, and if low concentrations of dextran could be accurately determined, one might be able to estimate the volume of circulating plasma by the dilution principle.

Suitable modifications of the anthrone determination of dextran as described by Bloom and Wilcox have been made to permit the measurement of serum concentrations below 100 mg. per cent with an accuracy of ± 2 per cent. Five normal male subjects were given 2.0 to 2.5 grams of a 6 per cent solution of dextran (N.R.C. Fraction No. 6, average mol. wt. 195,000) intravenously, and serum concentrations were determined at appropriate intervals from one minute to four hours. Rapid mixing was apparent, and the serum concentration fell at a slow exponential rate (1.8 per cent per hour). Renal excretion of this fraction was found to be negligible. The procedure was repeated two to four times on each individual, and the standard deviation of the variation from the individual's mean volume was ± 1.9 per cent (range ± .6 to ± 3.8 per cent). The average volume of distribution of dextran was 36.8 ml. per kg. of body weight.

High molecular weight dextran is distributed in a reproducible space. This space seems to be lower than plasma volume found with T-1824 or I^{131} albumin. However, dextran volume is comparable to the plasma volume found by using tagged cells and the venous hematocrit.

The Influence of Dietary Fructose on Intravenous Glucose Tolerance in Man. JAMES W. CRAIG, MAX MILLER,* and WILLIAM R. DRUCKER, Cleveland, O.

Hill, Baker, and Chaikoff (J. Biol. Chem., 1954, **209**, 705) concluded that the type as well as the quantity of dietary carbohydrate may influence the rate of utilization of subsequently administered glucose. Rats fasted for one day and then fed a diet whose sole carbohydrate was fructose for three days showed almost as great a diminution in tolerance for orally administered glucose as did rats fasted for four days; rats fed a diet whose sole carbohydrate was glucose served as controls. The altered tolerance was attributed to an impaired liver glucokinase activity, a manifestation of enzymatic adaptation to diet.

The present experiments were designed to determine whether or not dietary fructose altered glucose tolerance in man. Four normal young male subjects were fed a diet whose sole carbohydrate was 200 or 250 grams of fructose daily for three days, after which an intravenous glucose tolerance test was performed. Tolerance tests were performed in the same subjects after three days on a diet whose sole carbohydrate was the same quantity of glucose. Control tolerance tests were also obtained in the same subjects on a normal diet containing the same quantity of carbohydrate in mixed form. In these four subjects, fructose feeding produced no significant alteration in the intravenous glucose tolerance test.

In other human experiments it has been found that the increase in the glucose concentration of portal anastomotic vein blood after oral fructose administration is only a fraction of that found after equal amounts of oral glucose. The failure to find an altered glucose tolerance after fructose feeding suggests that liver glucokinase activity *in man* is not decreased by a lowered concentration of glucose in the portal vein blood. The explanation for starvation diabetes *in man*, therefore, awaits further experimentation.

A Procedure for Study of the Visceral Circulation in Man. JAMES W. CULBERTSON,* JOHN W. ECKSTEIN, and WALTER M. KIRKENDALL, Iowa City, Ia.

The physiopathology of a number of clinical circulatory disturbances remains incompletely understood because recorded clinical physiological observations have been fragmentary. Investigators have tended to concentrate on measurements of blood flow rate and pressure in a single regional circuit, often without concomitant estimation of total systemic blood flow rate (= MVCO). Previously reported combined procedures have related respiratory and circulatory phenomena.

Taking MVCO to represent pulmonary regional as well as total systemic blood flow, we have designed a practical procedure for measuring also the abdominal visceral (= renal + hepatic) blood flow rates separately but simultaneously during the same experimental session. Intracardiac and pulmonary arterial and "capillary" pressures are recorded before MVCO is determined (Fick principle). Then, with the venous catheter in the liver and a urethral catheter in the bladder, control estimates

of hepatic and renal plasma flow and splanchnic oxygen consumption are made by clearance methods. The constant intravenous infusion contains Bromsulfalein, sodium para-aminohippurate and inulin in a pharmaceutically and clinically compatible combination. These procedures require 90 minutes, a team of three physicians and < 150 ml. of blood for analysis. Provision is made for securing blood and urine samples for blank analysis, and adequate physiochemical equilibration time is allowed. In order to make estimates of renal and hepatic blood flow coincide the schedule conveniently staggers the collection times of blood and urine samples.

After completion of control observations acute physiological or pharmacodynamic changes may be induced deliberately and the experiment continued for another hour, with the initial series of observations repeated in reverse order.

Illustrative examples of the application of this procedure are available, depicting the acute hemodynamic changes seen following intravenous atropine in one instance and oral whiskey in another. The procedure is adaptable in various ways to suit individual investigators' needs.

Renal Conservation of Potassium during Electrolyte Restriction and the Effects of Sodium, Cold, and ACTH. T. S. DANOWSKI,* M. BLACK, R. MURTHA, and P. WIRTH, Pittsburgh, Pa.

Studies herein reported indicate that during potassium deprivation, in contradistinction to a widely held belief to the contrary, the kidney is capable of potassium conservation as precise as its conservation of sodium on sodium restricted regimens.

Transfer of grown rats to a diet free of electrolytes or containing only chloride resulted in a prompt and virtually parallel decrease in urinary sodium and potassium. The replacement of distilled drinking water with 0.5 or 1.0 per cent saline resulted in a 3 to 5-fold increase in urinary potassium excretion. Exposure of the rats to 5° C. temperatures also partially interfered with the renal conservation of potassium in response to a low electrolyte intake. Similar results were obtained with ACTH. The combination of cold and 1 per cent sodium chloride in the drinking water, or ACTH and oral 1 per cent sodium chloride, further increased urinary potassium output. In all instances, however, the potassium conservation response was still evident though less pronounced. These data indicate that in the rat deprived of electrolytes potassium conservation can be as effective as sodium conservation. Exposure to cold, administration of ACTH or the presence of sodium chloride in the intake interferes only partially with this response. The sodium and cold effects, and the sodium and ACTH effects, appear additive.

Assuming a similarity of response in rat and human kidneys, these findings suggest that the continued urinary loss of potassium during inanition which is one of the chief causes of potassium depletion in clinical situations is not attributable to an inherent inability of the kidney to conserve potassium. It is ascribable however to modifications in a fundamentally precise conservation response as a result of stress, sodium intake, and presumably other unidentified factors attendant upon illness.

Application of "The Doctrine of Original Antigenic Sin" to Immunization Against Influenza. FRED M. DAVENPORT and ALBERT V. HENNESSY, Ann Arbor, Mich. (Introduced by Thomas Francis, Jr.)

The "doctrine of original antigenic sin" epitomizes the discovery that, throughout life, antibody response to infection with influenza viruses is oriented to the dominant antigens of strains encountered in childhood. Three successive shifts in dominance of the antigens that characterize group A viruses have occurred. Consequently, antibody response to influenza A-prime in three successive cohorts of the population is predominantly to A-prime, A, or swine strains. Specific antibody increase to viruses encountered after childhood diminishes, yet the antibody spectrum broadens with age owing to repeated exposures. Concurrently, the attack rate falls. One objective of vaccination against influenza is to induce at all ages that composite of antibodies characteristic of older age groups. To ascertain an efficient method of achieving this goal, groups of 25 children, military recruits, or persons over 30 were vaccinated serially at two-week intervals with monovalent vaccines containing A-prime, A, or swine influenza viruses. Administration of these vaccines was alternated in "checker board" fashion. More than 500 individuals were studied. Antibody response was measured by hemagglutination inhibition in sera obtained before and two weeks after each vaccination. The results obtained demonstrate that reinforcement of the primary antibody characteristic of each age group is independent of the vaccine given. However, to achieve the maximal composite of antibodies, children require vaccination with swine and A, recruits with swine and A-prime, and persons over 30 with A and A-prime strains. A single vaccine composed of swine, A and A-prime viruses was tested and found to yield satisfactory antibody levels at all ages. These findings illustrate a cogent method for ascertaining the essential antigenic components of influenza vaccines, and provide a rational basis for alteration of current vaccine formulae.

An Electron Microscope Study of Sectioned Platelets and Megakaryocytes. QUIN B. DEMARSH, JEAN KAUTZ, and ARNO G. MOTULSKY, Seattle, Wash. (Introduced by H. S. Bennett).

Megakaryocytes and platelets obtained from human marrow biopsies, and platelets concentrated from peripheral blood have been fixed and sectioned sufficiently thin for electron microscope study. The preparations reveal many new structural features not seen in unsectioned material, and afford a promising approach for the study of platelet-megakaryocyte problems in health and disease.

The endoplasm of sectioned mature megakaryocytes displays numerous future platelet units, separated from each other by a three-dimensional array of platelet demarcation membranes, here described in the human for the first time. These membranes form from precursor structures, the platelet demarcation vesicles, found in the endoplasm of younger megakaryocytes. The vesicles line up and coalesce to form double layered plates. These fuse further to form the demarcation membranes, which come to enclose pockets of cytoplasm, each of which contains a few granules and constitutes a future platelet unit. Numerous additional granules can be seen elsewhere in the cytoplasm, along with mitochondria, Golgi material, large irregular vacuoles and endoplasmic reticulum. The ectoplasm is free of platelet demarcation membranes and other organelles.

Numerous small round or oval granules are also seen scattered throughout the hyaloplasm in sectioned platelets. The hyaloplasm is otherwise homogeneous, and is bounded by a relatively thick and sturdy platelet membrane. Most platelets are round or ovoid, but a few display spicules or agranular blebs at their borders.

Normal and abnormal megakaryocytes and platelets from sternal biopsy have been examined, as well as platelets from the peripheral blood of both normal patients and those with various bleeding disorders and blood dyscrasias. An attempt is being made to correlate submicroscopic morphology with clinical findings, in order to clarify especially the mechanism of platelet membrane formation in thrombocytopenic and thrombasthenic patients.

Studies on the Survival and Metabolic Activity of Platelets in Humans, Utilizing Radioactive Phosphorus. R. G. Desai, Walter Small, and Irma Mednicoff, Boston, Mass. (Introduced by Samuel H. Proger).

Studies have been made demonstrating the feasibility in humans of tagging platelets *in vivo* by the use of radioactive phosphorus.

Using sterile, siliconized apparatus, polycythemic platelet-rich plasma tagged *in vitro* with P-32 was injected into normal people, leukemic patients and the same polycythemic donor. Daily samples of blood from the recipients were collected, and platelet-rich plasma, platelet free plasma and washed platelets were prepared. By measuring the number of platelets and radioactivity in each sample, it was possible to calculate the radioactivity per million platelets. The apparent half survival time of platelets from polycythemic individuals injected in normals was 35 to 50 hours, while the same platelets in leukemics survived for 18 to 28 hours. The advantage of this method has been the requirement of smaller numbers of injected platelets, accuracy and reproducibility of measurements, and its applicability in various hematological states, *e.g.*, polycythemia, thrombocytosis, etc. It may also prove to be a method of studying *in vitro* the *in vivo* viability of platelets.

The results of the experiments have shown the uptake of P-32 by the platelets to be a function of temperature, incubation time, the amount of radioactivity and the tagging medium. The distribution of radioactivity in whole blood has been studied and indicates a direct relationship between metabolic activity of the cells and the phosphorus uptake. The integrity of the tagged platelets has been demonstrated by the clot reaction, thromboplastin generation, morphology and the Warburg respiration technique.

Kinetics of Human Adrenal Cortical Activity. V. Di-Raimondo, R. H. Orr, D. Island, and P. H. Forsham,* San Francisco, Calif.

When ACTH is given as an 8-hour i.v. infusion and hourly 17-hydroxycorticoids are determined, two phases in steroid excretion are noted: one of increasing excretion during the infusion and another of decreasing excretion following it. The effects of dose, time of day, and nature of ACTH on these phases of 17-hydroxycorticoid excretion were studied. For doses of ACTH of 1 USP Unit and above the acceleration in urinary excretion of 17-hydroxycorticoids was maximal. With smaller doses the acceleration varied with the dose. The duration of enhanced steroidogenesis was proportional to the dose. From minimal effective dose data the daily basal output of ACTH by the human pituitary is probably less than 1 USP Unit. Both acceleration and deceleration in urinary 17-hydroxycorticoid excretion were altered when infusions of ACTH were begun at 8:00 p.m. instead of 8:00 a.m. A diurnal variation in response to ACTH paralleling the spontaneous diurnal variation in adrenal cortical activity was demonstrated. This suggests that the diurnal variation is not controlled by endogenous ACTH secretion. Whereas sheep and hog ACTH accelerate steroidogenesis in the same manner the duration of enhanced steroidogenesis was more prolonged for the latter when comparing doses of equal ascorbic acid depleting capacity. This appears to be due to species differences. It is now possible, by studying the kinetics of steroidogenesis, to characterize any type of ACTH accurately in terms of steroidogenic potency, duration of action, and i.v./i.m. activity.

Effects of Sitosterol on Serum Lipids of Hypercholesterolemic Subjects. Charles H. Duncan and Maurice M. Best, Louisville, Ky. (Introduced by J. Murray Kinsman).

During the past two years the plant sterol, beta-sitosterol, has been administered to a number of human subjects, and its effect on the various serum lipid fractions noted. Of these, 10 meet the following criteria for inclusion in this report: control serum total cholesterol (Abell) of 260 mg. per 100 ml. or higher, and absence of those conditions in which elevation of serum cholesterol commonly occurs, diabetes mellitus, hypothyroidism, biliary obstruction, the nephrotic state, and essential hyperlipemia. Six to 8 grams of beta-sitosterol was administered immediately before the ingestion of food, the usual daily dosage being 20 to 25 grams. There was no

restriction as to type or amount of diet. The mean total period of observation was 45 weeks, including control (placebo) and treatment periods.

The administration of sitosterol was accompanied by a decrease in serum total cholesterol from a mean control level of 289 mg. per 100 ml. to 242 mg. per 100 ml. The decrease in cholesterol ranged from 21 to 104 mg. per 100 ml. The mean levels of the other serum lipid fractions were also decreased by sitosterol; total lipid from 967 to 832 mg. per 100 ml. (range minus 39 to minus 365); phospholipid from 356 to 325 mg. per 100 ml. (range plus 20 to minus 90); neutral fat from 324 to 260 mg. per 100 ml. (range minus 9 to minus 174). In 7 of the 10 patients lipoproteins as determined by the ultracentrifuge have also been studied, a trend toward reduction of S_f 3–100 classes being noted. The effects of sitosterol were noted by the end of one week, increased for the next several weeks, and were sustained throughout periods of administration up to one year. With the substitution of a placebo serum lipids returned to the pre-treatment range.

Natriuretic-Diuretic Effect of Angiotonin in Essential Hypertension. HARRIET DUSTAN, CARLOS NIJENSON, and A. C. CORCORAN,* Cleveland, O.

Observations on renal effects of angiotonin infusion were made during osmotic diuresis and hydropenia in 10 patients with essential hypertension and 1 normal subject. Angiotonin (hypertension) and renin induce natriuresis and diuresis in rats and rabbits, as renin does also in dogs with sectioned carotid sinus and aortic depressor (buffer) nerves, while renin does not in normal dogs (del Greco and Corcoran, 1954) and angiotonin does not in hydrated normal humans (Nickel *et al.*, 1954).

Angiotonin caused decreases in C_{PAH} and increases in filtration fraction in all, and associated decreases in $C_{Mannitol}$ in 3 of the patients. Urine flow and Na output were depressed in 5 patients and in the 1 normal subject; these include the 3 patients with decreased $C_{Mannitol}$. However, urine flow and Na output were increased in 5 of the patients, reaching nearly twice control rates in 1. The proportion of Na calculated as reabsorbed was decreased in these 5, while calculations of Tc_{H_2O} (free water reabsorption) demonstrate increases (0.2 to 1.4 ml. per min.). The diuretic effect of angiotonin in these patients is therefore osmotic and attributable to decreased Na reabsorption.

Two patients under treatment with ganglion-blockers at the time of test showed negative control rates of Tc_{H_2O} which became slightly positive after angiotonin. Abnormal water transport in these two presumably relates to a depressing effect of hexamethonium on maximum water reabsorption previously observed in hydropenic, osmotic diuretic dogs (del Greco and Corcoran, 1954).

The disparate effects of angiotonin on Na reabsorption in hypertensive patients may reflect differences similar to those mirrored by renal responses to renin in normal and buffer-nerve sectioned (neurogenic hypertensive) dogs.

An Evaluation of $Na_2Cr^{51}O_4$ as an Agent for the Determination of the Erythrocyte Life Span In Vivo *in Various Hemolytic States.* FRANKLIN G. EBAUGH, JR., CHARLES P. EMERSON,* and GERALD P. RODNAN, Bethesda, Md., and Boston, Mass.

Since there is a loss of Cr^{51} from labeled circulating erythrocytes in normal individuals, the validity of this technique has been studied in the hemolytic anemias by selective agglutination and radioactive Cr^{51} counting. The rate of elution of Cr^{51} from circulating normal donor cells in recipients with hemolytic disease and from abnormal red cells (sickle cell anemia and congenital spherocytosis) transfused into normal recipients was no faster than from normal donor cells in normal recipients, *i.e.*, 52 to 97 days—for half the Cr^{51} to disappear from circulating RBC. It was not possible to estimate red cell survival by external monitoring of the gamma radiation emitted from the patient because Cr^{51} which left the blood on any given day had a tissue phase before eventual urinary excretion. During the first 24 to 48 hours after intravenous injection of *in vitro* tagged RBC or aqueous $Na_2Cr^{51}O_4$, the radioactivity of the blood dropped 2 to 10 per cent, a decrease greater than that solely due to RBC destruction in the period. Because this decrease is observed for RBC tagged *in vivo* as well as *in vitro*, for non-chromium labeled donor cells as measured by selective agglutination, and since no such increase is seen in the elution rate of Cr^{51} from donor cells immediately following transfusion, this rapid initial fall may not be due to cell damage or loss of Cr^{51} from circulating cells but due to failure of donor cells or *in vivo* tagged cells to fully equilibrate with the entire red cell mass during this period of time. Whole blood should be used for Cr^{51} survival studies since RBC washed in saline had a mean survival time (corrected for elution of Cr^{51}) of 93 days in normal men compared with 127 days for unwashed cells.

Basic Amino Acids in Experimental Potassium Deficiency. ROBERT E. ECKEL and JAMES E. C. NORRIS, Cleveland, O. (Introduced by Reginald A. Shipley).

Since Christensen *et al.* have shown that basic amino acids affect the distribution of ions between cells and the extracellular fluid, the role of these acids in the electrolyte disturbance of K depletion was studied. In 100 gm. rats where growth was arrested by four weeks of a K deficiency diet, mean data for groups of six rats showed total basic amino acids (BAA) and 1(+)lysine attained concentrations of 9.89 and 7.74 mM per Kg. intracellular water (ICW), respectively, compared to levels of 0.82 and 0.73 mM per Kg. ICW in paired weighed controls and 1.52 and 1.68 mM per Kg. ICW in growing controls. Lysine also accumulates to the same degree in DOCA produced K deficiency despite liberal K intake and normal growth.

The feeding of 1(+)lysine HCl accelerates the development of K deficiency on a K deficient diet and further increases intracellular lysine to 25 mM per Kg. ICW. In pair fed animals on the same diet containing K, the

intracellular Na increases significantly from 15.7 to 22.2 mM per Kg. ICW and lysine increases to 10.5 mM per Kg. ICW. Total cations are elevated above normal in both cases.

Because of the very small changes in serum BAA and lysine concentrations, the lysine gradient across the muscle cell membrane increases in K deficiency. About half the increased hydrogen and ammonium ion excretion during recovery from K deficiency observed by Cooke *et al.* would occur if the BAA present in K deficiency were metabolized in the recovery process. The known metabolism of 1(+) lysine HCl to ammonia in acidosis and to urea in the absence of acidosis furnishes an explanation of the urinary ammonia findings of Cooke. Lysine thus apparently has a role as a labile muscle cation analogous to the role of ammonia, bicarbonate, and citrate in the urine.

The Metabolism of Plasma Lipoproteins. HOWARD A. EDER* and DANIEL STEINBERG, Bethesda, Md.

The metabolism of plasma lipoproteins in the rabbit was studied by labeling the proteins with alanine-1-C^{14} and the phospholipids with P^{32}. Plasma lipoproteins were fractionated by ultracentrifugal flotation into lipoproteins with densities less than 1.063, lipoproteins with densities between 1.063 and 1.21 and residual proteins with densities greater than 1.21.

After injection of alanine-1-C^{14} the $D < 1.063$ lipoproteins attained maximal specific activity at 6 hours. Activity then fell precipitously so that by 20 hours it was less than 25 per cent of the maximal activity; thereafter it decreased more slowly. The D 1.063–1.21 lipoproteins attained specific activities only one-third as great and the maximal activity was found after 6 hours. The initial rate of decrease in activity was considerably slower and the $t_{1/2}$ of the slow phase was about 12 days. The curve for the $D < 1.21$ fraction, the bulk of the plasma proteins, was very similar.

In other experiments the various fractions were labeled by alanine administration and each of the three fractions was administered to a different recipient rabbit. The labeled $D < 1.063$ lipoproteins disappeared with great rapidity while the labeled $D < 1.063$–1.21 lipoproteins disappeared more slowly. The data do not permit conclusions regarding the interconversion of the proteins of one class to another. Similar experiments with protein of higher activity are in progress.

That the rapid disappearance of the $D < 1.063$ lipoproteins was not due to the presence in this fraction of chylomicra with high metabolic activity was demonstrated by experiments in which the very low density components were analyzed separately.

Phospholipid reached maximal activity by 15 hours. The specific activities in the different fractions were virtually identical. This can be explained by the observation that phospholipid rapidly transfers from one lipoprotein fraction to another even *in vitro*.

These experiments demonstrate that the various lipoprotein proteins differ in their metabolic patterns.

The Effects of Steroid Hormones on Anabolism and Catabolism of Normal Tissues and Lymphoid Tumors in Humans on Protein-Free Diets. LEONARD P. ELIEL* and ROBERT P. HEANEY, Oklahoma City, Okla.

Maximal reduction of protein anabolism, by administration of protein-free, calorically adequate diets has been employed to assess the effects of steroid hormones on anabolism and catabolism of normal and neoplastic tissues in man. In three studies, after achievement of stable, minimum nitrogen excretion, cortisone acetate, 200 to 400 mg. per day p.o., or Testosterone propionate, 50 mg. per day i.m., was administered for one to three weeks. The balances of nitrogen and electrolytes were determined. One patient had no lymphoid tumors, the other had chronic lymphatic leukemia with generalized adenopathy and splenomegaly.

Amino acid deprivation was invariably associated with losses of nitrogen, phosphorus, and potassium in proportions consistent with catabolism of normal tissue. The leukemic patient failed, however, to demonstrate significant changes in lymphoid tumor mass, either by clinical measurement or by altered ratios of the phosphorus to nitrogen balances.

The patient without lymphoid tumors demonstrated, upon exhibition of cortisone, a minimal rise in nitrogen excretion, averaging 0.1 gm. per day. The patient with leukemia, however, demonstrated almost complete disappearance of enlarged lymph nodes and moderate spleen shrinkage. There was a rise in nitrogen excretion averaging 1.36 gm. per day, 60 per cent of which was calculated to arise from loss of lymphoid tumors. Administration of Testosterone to the same patient, during a second study under identical conditions, failed to produce any reduction in the negative nitrogen balance.

The data suggest that cortisone acetate does not under these conditions accelerate catabolism appreciably, since it increases nitrogen excretion from normal tissues but slightly when anabolism is reduced markedly. The data also suggest that Testosterone propionate does not act by inhibiting catabolism since it failed to diminish nitrogen excretion when anabolism was maximally reduced. Continued anabolism of lymphoid tumors during protein deprivation is suggested by their failure to shrink and their usual response to administration of cortisone.

Studies on the Physiologic Role of Glucagon (Hyperglycemic Factor). H. ELRICK, C. J. HLAD, JR., T. WITTEN, T. M. BOW, and A. SMITH, Denver, Colo. (Introduced by G. S. Gordan).

Glucagon causes hyperglycemia and a decrease in liver glycogen, apparently by accelerating liver glycogenolysis. Beyond this, little is known about its action or its function in the body. The purpose of this paper is to describe some experiments designed to elucidate the action of glucagon on glucose utilization and to propose a theory as to its physiologic role.

Glucose was administered intravenously by constant infusion pump for periods of two hours to 38 normal

adults under controlled basal conditions. The effects of glucagon and/or insulin on arterial (capillary) and venous blood sugar were studied. It was found that glucagon consistently increased A-V glucose differences markedly, whereas insulin did not. Both hormones together (1) increased A-V glucose differences beyond what glucagon alone did and (2) maintained blood sugar levels between the elevated or depressed levels resulting from each alone. These results have been confirmed in the pancreatectomized dog.

The increases in A-V glucose differences were far greater than those predicted or actually obtained by elevated glucose levels *per se*. Since the experimental technique used minimizes changes in blood flow, it is felt that the A-V differences observed were proportional to peripheral glucose utilization.

The theory is proposed that glucagon and insulin function as a team to enhance peripheral glucose utilization and, simultaneously, maintain relative constancy of blood glucose and liver glycogen levels. Since glucagon action is rapid and short-lived, the postulated action probably serves as an emergency function. Because insulin and glucagon have opposing effects on blood glucose and liver glycogen, each is ideally suited to complement the action of the other in maintaining constancy of these two reservoirs of body glucose in the face of accelerated glucose utilization. The observed effects of glucagon are not due to hyperglycemia *per se* or to a secondary release of insulin.

The Relationship of Serum Myeloma Proteins and Bence-Jones Proteins, Particularly with Respect to Their Methionine Content. RALPH L. ENGLE, JR., LILA A. WALLIS, and BERNARD UDIN, New York, N. Y. (Introduced by Paul Reznikoff).

The relation of Bence-Jones protein to the abnormal serum protein component in patients with multiple myeloma remains unknown. Bence-Jones proteins have been variously reported to contain either no methionine or amounts corresponding to less than two residues per molecule of protein. Accordingly, the aminoacid composition of the serum myeloma proteins and Bence-Jones proteins occurring simultaneously in two patients was determined. In addition, similar analyses were made of five other Bence-Jones proteins.

The proteins were isolated by thick filter paper and starch block zone electrophoresis, and were examined for aminoacid composition by two dimensional paper chromatography. The sensitivity of the technic used was sufficient to detect one residue of methionine per Bence-Jones protein molecule of 44,000 molecular weight.

The only qualitative difference in the aminoacid content of the proteins studied was the absence of detectable amounts of methionine in five of the seven Bence-Jones proteins. In one of the two patients having both serum myeloma and Bence-Jones proteins, methionine was found in both proteins in about the same concentration as in normal gamma globulin. However, chemical differences in these two proteins were found by N-terminal aminoacid analysis (method of Edman). Aspartic acid was the predominant end residue for the serum protein, while there was no N-terminal aminoacid for the urinary protein by this technic. In the other patient, the Bence-Jones protein contained no detectable methionine; yet the serum myeloma protein contained the usual amount of methionine.

The results suggest that most Bence-Jones proteins contain only trace amounts of methionine. Other Bence-Jones proteins, however, contain about the same per cent methionine as normal gamma globulin. The chemical differences between the Bence-Jones and the serum myeloma proteins of the same patient suggest that these proteins are not directly related.

Folic Acid and Citrovorum Factor Metabolism in Gout. WILLIAM W. FALOON and SHIRLEY FEIGEL, Syracuse, N. Y. (Introduced by Eugene L. Lozner).

Citrovorum factor has been demonstrated to function as a transformylating medium in purine synthesis and in direct uric acid synthesis. The conversion of pteroylglutamic acid to citrovorum factor and the urinary excretion of folic acid (Strep. Fecalis growth stimulating factors) and citrovorum factor have been studied in gouty patients.

Urinary excretion of citrovorum factor (CF) and folic acid (FA) was studied during four-day periods of oral administration of 10 mgm. of pteroylglutamic acid (PGA) daily. The average daily urinary excretion of CF during these periods in eight gouty patients was 18 gamma (range 6 to 29); in 10 healthy normals 38 gamma (range 18 to 65); and on four hospitalized non-gouty individuals 36 gamma (range 17 to 60). The differences in CF excretion by gouty and normal individuals were statistically significant, (p less than 0.01). Blood ascorbic acid levels were lower in gouty patients (average 0.42 mgm. per cent) than in normals (0.94 mgm. per cent) or non-gouty patients (0.71 mgm. per cent). No correlation was found, however, between blood ascorbic acid and CF excretion in patients or in normals. Continued administration of PGA for periods longer than four days resulted in increasing CF excretion to levels comparable with normals. In contrast to the CF excretion, the average FA excretion was nearly identical in gouty patients and healthy normals, being 4500 gamma and 4300 gamma per day, respectively. PGA administration produced no consistent change in serum uric acid or urate excretion.

Ascorbic acid given orally during PGA administration yielded increased CF excretion in the three gouty patients so studied. Salicylates, phenylbutazone, and colchicine produced no consistent change in CF excretion.

The decreased excretion of CF during administration of FA in gouty patients may be explained by several hypotheses which will be discussed. The most probable of these are decreased body stores or increased utilization of CF.

Studies of The Influence of Adrenal Steroids on Pyruvate Metabolism in Man. THOMAS F. FRAWLEY, Albany, N. Y. (Introduced by Walter S. McClellan).

Blood glucose determinations and glucose tolerance tests may not reveal early alterations in carbohydrate metabolism due to adrenal steroids. Studies were undertaken to ascertain the effect of so-called carbohydrate-active adrenal steroids on certain intermediaries of carbohydrate metabolism. Consequently some of the metabolic reactions involving glucose, pyruvate, lactate, phosphorus, and potassium have been studied.

Ingle's studies in animals using constant intravenous injections of adrenal steroids prompted an adaptation of this technique to clinical studies in man. Constant infusions of hydrocortisone were administered intravenously (12 to 25 mg. per hour) over a seven-hour period—as this simulates probably the manner in which the adrenal cortices secrete their hormones. At the third hour, glucose (0.5 Gm. per Kg. body weight as a 20 per cent solution) was administered intravenously for twenty-five minutes. In normals the glucose curve obtained during intravenous hydrocortisone did not differ significantly from control curves. Pyruvate was slightly more elevated and prolonged. Superimposing a stressor (pyromen) elevated and prolonged the pyruvic acid response without significantly changing the glucose tolerance. During the stress of hypoglycemia the pyruvic acid response to hydrocortisone (25 mg. per hour) was observed to be greater than with corticosterone administered similarly. Patients receiving corticotropin or corticosteroid therapy showed an elevated fasting blood pyruvic acid prior to a change in blood glucose. The tolerance for fructose was measured since fructose phosphorylation is independent of insulin and presumably the anti-insulin effect of adrenal steroids. Intravenous fructose (0.5 Gm. per Kg. in 10 per cent solution) during intravenous hydrocortisone (12.5 mg. per hour) gave normal tolerance values and the normal rise in pyruvic acid was somewhat prolonged.

Adrenal steroids can produce an alteration in blood pyruvic acid independently without any appreciable change in blood glucose. This effect like that occurring with potassium and phosphorus suggests certain fundamental differences between so-called "steroid-diabetes" and diabetes mellitus.

The Effect of Inflammation on the Utilization of Erythrocyte and Transferrin Iron for Hemoglobin Synthesis. E. J. FREIREICH, A. MILLER, C. P. EMERSON,* and J. F. ROSS,* Boston, Mass. and Los Angeles, Calif.

The pathogenesis of the anemia associated with inflammatory disease remains an enigma. Although inflammation produces marked changes in iron metabolism, none of the demonstrated changes have explained the development of anemia. As an approach to this problem, we have studied the re-utilization for hemoglobin formation of radioiron released from transfused nonviable radioiron labelled donor erythrocytes in normal dogs and in dogs in whom sterile abscesses were produced by injections of turpentine. Similar observations were made following intravenous injection of tracer amounts (5 micrograms) of transferrin-bound radioiron. Although both control and "abscessed" animals rapidly incorporated 75 per cent to 85 per cent of transferrin-bound iron into newly formed hemoglobin within 10 days the re-utilization of "erythrocyte iron" was markedly decreased in rate and amount in the "abscessed" animals (only 10 to 20 per cent in 5 days, and 45 to 50 per cent in 30 days), in contrast to efficient and rapid utilization by the controls (45 to 55 per cent in 5 days and 70 to 80 per cent in 30 days).

These studies indicate that inflammation does not impair the utilization for hemoglobin formation of very small (? physiological) tracer doses of transferrin-bound plasma iron, but that inflammation does markedly interfere with the normal mechanism of catabolism and reutilization of erythrocyte iron. Since hemoglobin synthesis normally is dependent upon re-utilization of "erythrocyte iron," relative decreased delivery of this "building stone" to the plasma would result in a decrease of serum iron concentration and would contribute to the development of anemia.

Study of Hepatic Lymph in the Intact Animal. MEYER FRIEDMAN,* SANFORD O. BYERS, and RAY H. ROSENMAN, San Francisco, Calif.

Collection and analysis of hepatic lymph offers an unique method by which the intercellular fluid of the liver may be studied because of the probable chemical identity of the two fluids. By means of a new method, hepatic lymph was collected from normal rats and from those exposed to various experimental procedures.

The average lymph flow of six normal rats was found to be approximately 0.4 cc. per hour with an increase of 300 per cent after ligation of the bile duct. The average cholesterol, protein and cholic acid concentrations were always less than those of plasma, being 52, 75, and 74 per cent, respectively, of the plasma values. The average lymph glucose, however, was found to be about twice that found in plasma. The concentration of the latter, however, appeared to vary with the rate of flow.

The cholesterol content of lymph unlike that of bile appears to be independent of the rate of hepatic synthesis of cholesterol for when the latter is increased by thyroid administration, no change occurs in lymph cholesterol despite the marked increase observed in biliary cholesterol. Conversely, experimental elevation of plasma cholesterol in the normal rat by intravenous administration of hypercholesteremic serum is followed by a marked increase in lymph but not in bile cholesterol.

It is probable that with the possible exception of glucose, the constituents of hepatic lymph are chiefly derived from and their concentration determined by the plasma content of these same constituents. Analysis of this fluid, therefore, may offer a new means of studying the diffusion pattern of various plasma solutes from the hepatic vasculature.

The Effects of Increased Ventilation on the Work of Breathing in Patients with Pulmonary Emphysema and in Normals. MORTON GALDSTON* and JACK GELLER, New York, N. Y.

The effects of increased ventilation induced by Diamox® (2.2 to 6.3 mgm. per Kg. every 6 hours) and/or single 0.5 G. intravenous Aminophylline doses in emphysematous patients with and without carbon dioxide retention, and in normals, were analyzed by plotting alveolar pCO_2 mm. Hg and oxygen consumption (ml. per Min.) against ventilation (L. per Min.) and CO_2 output (ml. per Min.) against alveolar ventilation (L. per Min.).

In two advanced emphysematous patients alveolar pCO_2 fell from 54 to 44 mm. Hg and from 62 to 45 mm. Hg, respectively, when ventilation increased 2.0 to 2.7 L. per Min. In the former, this was accompanied by increased CO_2 output and oxygen consumption. Further ventilatory increase, accomplished only in the latter (4.0 L. per Min. maximum attained), failed to decrease alveolar pCO_2 because increased rate of CO_2 production due to increased work of breathing reached the maximum which the alveoli could ventilate effectively.

Two moderately emphysematous patients attained minimal alveolar pCO_2 levels (32 and 28 mm. Hg) with 3 to 4 liters ventilatory increase, without a change in metabolic rate. No further alveolar pCO_2 fall occurred, even with a 5½ to 9½ L. per Min. increase in ventilation. In one, with a markedly restricted maximum breathing capacity, this was accompanied by dyspnea, increased oxygen consumption and carbon dioxide output.

One of three normals attained a minimal alveolar pCO_2 of 35 mm. Hg with a ventilatory rise of only 2 liters. Additional ventilatory increase of 1 L. per Min. was accompanied by a greater metabolic rate and no change in alveolar pCO_2. The remaining two, whose alveolar pCO_2 fell to 34 and 24 mm. Hg, with 3.5 to 5 L. per Min. ventilatory increases, did not exhibit a minimum pCO_2 value or an elevation in metabolism.

These studies demonstrate that increase in ventilation beyond an optimal point no longer results in more effective alveolar ventilation because of increased work of breathing and may be detrimental.

The Mechanism of Cardiac Changes Observed in Uremia. M. M. GERTLER, J. KREAM, and J. W. HYLIN, New York, N. Y. (Introduced by B. S. Oppenheimer).

The concomitant and sequential cardiac changes in the early and terminal stages of uremia as manifested by the electrocardiogram closely parallel the cardiac changes often observed with high levels of serum potassium and/or stimulation of the cardiac vagi. These changes cannot be attributed solely to changes in intracardiac serum electrolytes for the relationship between intracardiac electrolytes and electrocardiographic measurements has not been firmly established. Because of the known association between vagal stimulation and potassium concentration another possible mechanism for these changes in uremia was considered, namely, a study of the cholinesterase system in cardiac musculature.

Uremia was produced in eight male dogs by bilateral ligation of the ureters. Three animals were treated actively with ionic exchange resins while five were permitted to continue the usual uremic course. Electrocardiograms were taken daily on the animals and when death appeared imminent, the animals were sacrificed.

The hearts were quickly removed and frozen. Homogenates of ventricular muscle were made and analyses were performed for cholinesterase activity by Ammon's method. It was found that the uremic animals had an average cholinesterase activity of 2.8 units (range 2.1 to 3.3) while in the normal animals the average was 4.4 units (range 3.5 to 5.6) as indicated by Q CO_2 values.

The significance of these findings as a possible explanation for the cardiac changes observed in uremia will be presented.

Myoalbumin, Plasma Albumin and Interstitial Fluid in Human and Rabbit Muscles. DAVID GITLIN, DORIS NAKASATO, and WILLIAM R. RICHARDSON, Boston, Mass. (Introduced by James L. Gamble).

The functional significance and biochemical properties of myoalbumin, a protein found in aqueous extracts of muscle, are largely unknown. Myoalbumin has never been isolated in its native state and has been characterized solely by solubility in aqueous solutions, resistance to denaturation and electrophoretic mobility. The superficial resemblance of these properties to those of plasma albumin prompted us to investigate the relationship between myoalbumin and plasma albumin in human and rabbit muscles employing radioactive and immunochemical methods in association with electrophoretic fractionation.

It is possible to demonstrate that: 1) The concentration of plasma albumin in the interstitial fluid of normal human and rabbit muscles is, respectively, about one-fifth and one-fourth that in the circulation; and 2) the mass of albumin in the interstitial fluid is approximately equal to that in the vascular system. These relationships are not specific for albumin but are apparently true for other plasma proteins, such as γ-globulin, which we have been able to estimate. The extravascular presence of such relatively large amounts of plasma protein was predicted by Drinker's hypothesis that the protein content of lymph fluid is representative of that in the interstitial fluid and is in accord with Starling's hypothesis of the capillary.

It has also been found, in correlation with immunohistochemical data, that myoalbumin for the most part is not derived from the muscle fiber and is simply plasma albumin originally present in the interstitial fluid and residual blood plasma in the muscle sample. The paradoxical increase in myoalbumin which accompanies denervation atrophy is due primarily to a relative increase in the extracellular mass of plasma albumin.

The Biosynthesis of the Purine Ring. D. A. GOLDTHWAIT, G. R. GREENBERG, and R. A. PEABODY, Cleveland, O. (Introduced by H. S. Ginsberg).

The purine nucleotides occupy a key role in the biosynthesis of many cofactors, of nucleic acids, and of adenosine triphosphate (ATP). Since Aminopterin and azaserine, both effective tumor chemotherapeutic agents, block the synthesis of purine nucleotides, an understanding of the mechanism of purine biosynthesis should lead to the development of more oncolytic agents. The synthesis of the nucleotide inosinic acid has been studied in an acetone powder extract of pigeon liver. With the proper additions and the omission of $KHCO_3$, early precursors of inosinic acid accumulate. These have been isolated and purified by paper and ion-exchange chromatography. One has been assigned the structure glycine amide ribotide (I), while the other (II) is similar but contains a formyl group. These compounds have since been shown in another laboratory to accumulate in the presence of azaserine. In the sequence of reactions leading to the synthesis of these compounds, ribose-5-phosphate reacts with ATP to form ribose-1-pyrophosphate-5-phosphate (PRPP). This compound has been shown to react with glutamine, but the product is still unknown. PRPP, glutamine, glycine, and ATP plus the Dowex treated dialyzed enzyme react to form I. If formate and tetrahydrofolic acid or citrovorum factor are included II is synthesized. Aminopterin blocks the reduction of folic acid to tetrahydrofolic acid and thereby inhibits the transfer of formate *via* tetrahydrofolic acid to I and also to imidazolecarboxamide ribotide (III) to form inosinic acid. N^{10}-formyltetrahydrofolic acid and not citrovorum factor is the active transformylating cofactor in this system. The reactions between II and III which must include ring closure and addition of two nitrogens and the carbon of CO_2 are unknown. Studies on the mechanism of fixation of CO_2 into the purine ring suggest that Krebs cycle intermediates are not involved.

The Detection of Pulmonary Congestion Utilizing the Valsalva Maneuver. RICHARD GORLIN, JOHN H. KNOWLES, and CLIFFORD F. STOREY, Portsmouth, Va. (Introduced by Eugene C. Eppinger).

The immediate effect of the Valsalva maneuver is to impede thoracic venous inflow with resultant fall in systemic arterial pressure during the strain. With release of the strain, systolic and pulse pressures both increase; *i.e.*, the "overshoot." Both auscultatory and directly recorded systemic arterial pressures have been measured during the Valsalva maneuver in 55 patients, with particular attention directed toward changes during straining. The time of and rate of fall of arterial pressure during strain was altered by body position and certain disease states. Arterial pressures decreased immediately during the strain in standing normals, and in pregnancy, pulmonary disease and mild mitral stenosis. Recumbency resulted in larger pulse pressures in all groups. Recumbency with feet elevated resulted in maintenance of arterial pressure from 2 to 3 seconds in some normals to longer periods in patients with minimal pulmonary congestion. In severe mitral stenosis, constrictive pericarditis, and left ventricular failure, arterial pressure was sustained seven seconds or more in all positions with intensification in recumbency. During the maneuver peripheral venous pressure rose in linear fashion but never exceeded the positive pressure of straining until after the first eight seconds. The poststraining overshoot was absent in general when arterial pressure was sustained during straining. Sustained arterial pressures during the maneuver correlated with prolonged circulation time, decreased vital capacity and total lung volume. Ausculted blood pressure was sufficiently accurate to differentiate between the response with congestion and in the normal and to detect the presence or absence of an overshoot. As such the arterial pressure response to the Valsalva maneuver was utilized as a simple clinical test for the detection of pulmonary congestion. Cardiac dyspnea has been separated from that due to pulmonary disease; pulmonary congestion has been detected where unsuspected; and the effects of cardiac surgery on pulmonary congestion easily evaluated.

Continuous Recording of Thoracic-Aorta Flow in Man. JOSEPH B. GRACE, WILLIAM P. CROWLEY, IRWIN J. FOX, and EARL H. WOOD,* Rochester, Minn.

The dilution technic using constant-rate injection of the indicator is the closest approach to an acceptable method for continuous recording of cardiac output in man. The period during which instantaneous flow can be recorded is, however, limited by recirculation of indicator. If, however, the injection is made into the thoracic aorta, only recirculated indicator reaches the aortic-arch arteries and in identical concentrations to the recirculated indicator which is "contaminating" thoracic-aorta blood as the injection continues. Under these conditions the concentration of recirculated indicator can be recorded from a radial artery and used as a baseline for measurement of injected indicator on its first passage by the sampling site (femoral artery) throughout any duration of constant-rate injection that may be desired. Then if (1) uniform mixing of an indicator injected in the thoracic aorta occurs, (2) the rate of injection is known, and (3) the indicator concentration is recorded continuously at a femoral and radial artery, the instantaneous blood flow down the thoracic aorta can be obtained for the duration of the injection. Constant-rate 60-second injections of T-1824 were made *via* an 80-cm. (0.5 I.D., 1.0 mm. O.D.) catheter into the thoracic aorta while its concentration was recorded from the radial and femoral arteries of 21 subjects. Erratic results for thoracic aortic flow (average: 64, range: 46 to 90 per cent of cardiac output [direct Fick]) indicated that uniform mixing was not obtained. With an end-sealed catheter having six (0.1 to 0.2 mm. diameter) radially spaced holes near the tip and an injection rate of 30 ml. per minute, thoracic aorta flow ranged from 69 to 77 per cent of cardiac output. Flows recorded

during Valsalva and tilting maneuvers showed similar directional changes to mean arterial (subclavian) pressure.

Relationship Between the Stomach and the Adrenal Cortex. SEYMOUR J. GRAY,* LEWIS J. KRAKAUER, RAMON VILLAREAL, COLIN G. RAMSEY, and ROBERT REIFENSTEIN, Boston, Mass.

The present report encompasses evidence of a relationship between gastric activity and adrenal cortical function.

Adrenocorticotrophic hormone or the adrenal glucocorticoids (Compounds E, F, B) produce a considerable increase in gastric juice acid and pepsin both in human subjects and in Heidenhain pouch dogs. The chloride concentration of the gastric juice increases significantly and is accompanied by a fall in sodium and potassium concentration. A single injection of 40 units of intramuscular ACTH to Heidenhain pouch dogs elicits a significant gastric response within four to six hours. A more prolonged stimulation is necessary in humans. Removal of the antrum and vagectomy do not alter the gastric response to the adrenal steroids in humans or experimental animals, indicating a direct action of the glucocorticoids upon the stomach. Mineralocorticoids, testosterone, and estrogens are ineffective.

The urinary uropepsin excretion, as a measure of gastric peptic activity, has been correlated with the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids to investigate the relationship between gastric and adrenal activity.

A diminished uropepsin excretion paralleled by a decrease in urinary steroid excretion was observed in twelve patients with untreated Addison's disease (P less than 0.01). With replacement glucocorticoid therapy the uropepsin returned to normal. Conversely in twelve patients with adrenal hyperfunction (Cushing's disease) a significant increase in uropepsin excretion was accompanied by an elevation in the urinary corticoids (P less than 0.01).

The relationship between the stomach and the adrenal gland is further borne out by a significant correlation of uropepsin and adrenal corticoid excretion in normal subjects. This correlation is significantly implemented during the administration of ACTH, and is of a greater order of magnitude than that observed in the unstimulated normals.

The Biophysics of the Variants of Sickle Cell Disease. ROBERT C. GRIGGS and JOHN W. HARRIS, Cleveland, O. (Introduced by Frederick C. Robbins).

The mechanisms have been investigated concerning the intracellular content of S-hemoglobin and the oxygen tensions at which the erythrocytes sickle, as causally related to the severity of the clinical and hematological manifestations of the various syndromes associated with sickle hemoglobin.

Six patients with sickle trait, six with sickle-C disease, three with sickle-Thalassemia disease and eight with sickle anemia were studied. At various oxygen tensions observations were made on tactoid formation in hemoglobin solutions, and on the number of sickled cells, erythrocyte mechanical fragility and viscosity of whole blood samples. For a sickle hemoglobin concentration of 90 to 100 per cent, as observed in the erythrocytes of the clinically severe sickle anemia, an oxygen tension as high as 60 mm. of mercury produces the biophysical changes *in vitro* of increased numbers of sickled cells, increased mechanical fragility and increased whole blood viscosity. For sickle hemoglobin concentrations of 50 to 80 per cent, as observed in the clinically intermediate sickle-C and sickle-Thalassemia diseases, a lower oxygen tension, 40 mm. of mercury, is required to produce comparable biophysical changes. Both of these oxygen tensions can occur physiologically. However, for sickle hemoglobin concentrations of 24 to 45 per cent, as observed in the clinically benign, non-anemic sickle cell trait, the oxygen tension must be extremely reduced, to a non-physiologic level of 10 mm. of mercury, to produce the above biophysical changes.

The formation of small tactoids in concentrated hemoglobin solutions and the increased mechanical fragility occurred at higher oxygen tensions than are required to effect sickling. These findings suggest that small intracellular tactoids, not large enough to distort the intact cell produce alterations leading to increased mechanical fragility.

These observations indicate that the degrees of anemia and circulatory alterations found in the variants of sickle cell disease are related to the intracellular content of S-hemoglobin and the biophysical changes produced by low oxygen tensions.

A Method for Estimating the Inferior Vena Caval Blood Flow in Man. JACOB GROSSMAN, SHIRLEY RUBLER, HELEN B. HOROWITZ, and RAYMOND E. WESTON,* New York, N. Y.

The modified Fick principle previously employed in the laboratory for the measurement of cardiac output without gas analysis, using PAH as the test substance, has been adapted for the estimation of lower inferior vena caval blood flow. The method involves placing the tip of a double lumen catheter into the right renal vein with the opening of the other lumen in the inferior vena cava above both renal veins. From the plasma concentration of PAH in arterial ($[PAH]_A$) and caval ($[PAH]_{VC}$) blood samples, simultaneously withdrawn at intervals during determinations of renal plasma flow (RPF), the lower inferior vena caval blood flow (LVCF) may be calculated from the following formula:

$$LVCF = RPF \frac{[PAH]_{VC} - [PAH]_{RV}}{[PAH]_A - [PAH]_{VC}} \times \frac{1}{1 - Hct}$$

where $RPF = C_{PAH}/E_{PAH}$, $[PAH]_{RV}$ = renal venous plasma PAH concentration, and Hct = hematocrit.

The estimated LVCF includes largely the venous drain-

age from both lower extremities including the gluteal region, the left gonad and adrenal gland, and, in females, the pelvic reproductive organs.

In eight normal and eight cardiac subjects, the estimated LVCF ranged from 25 to 33 per cent of the cardiac output which was measured during the procedure, although not simultaneously. In patients with decreased cardiac outputs, LVCF was proportionately reduced. When the cardiac output was increased by exercise or pyrogen reaction, a proportionate increase in LVCF occurred. The assumptions and errors inherent in the methods will be discussed.

Nucleic Acid Metabolism in Chronic Leukemia. L. D. HAMILTON, New York, N. Y. (Introduced by Joseph H. Burchenal).

Adenine-8-C^{14} was given intravenously to patients with chronic lymphocytic and chronic myelocytic leukemia. Ribose nucleic acid (RNA) and deoxyribose nucleic acid (DNA) were separated from cells collected over periods of up to 670 days, and the radioactivities of the separated individual purines determined.

In the granulocytic cells the incorporation of adenine as such into the DNA was as extensive as into the RNA, and there was extensive transformation of adenine into RNA and DNA guanine. The retentions of isotope in the adenine and guanine of RNA and DNA were similar—consistent with data from other rapidly dividing tissues. In contrast, in lymphocytes, the incorporation of adenine into DNA (and its transformation into guanine) was less extensive than into RNA.

The decline in activities of both RNA and DNA of lymphocytes could be divided into two phases—an initial more rapid component (DNA adenine of roughly 18 days half-time), and a residual slow component showing a half-time of the order of 300 days. In granulocytes there was no such prolonged retention of the isotope in the polynucleotide purines—activity could not be demonstrated beyond 140 days. The decline in activity of DNA adenine in the first component had a half-time of approximately 9 days, and the second component with a somewhat longer half-time, was a relatively insignificant proportion of the total.

These results suggest that leukemic granulocytes survive for shorter times than leukemic lymphocytes; we cannot yet tell whether the results in lymphocytes represent the survival of leukemic lymphocytes for very long periods, or a specific re-utilization by lymphocytes of large fragments of nucleic acid of their predecessors. The latter interpretation is favored by the relative insignificance of the second component in granulocytes, and the morphological evidence compatible with re-utilization —phagocytosis of lymphocytes by reticulum cells in lymph nodes. Such re-utilization could be a method for blood cell differentiation following transformation of the primitive multipotential cell by phagocytosed DNA.

The Relationship of Platelets to the Serum Potassium Concentration. ROBERT C. HARTMANN and SHERMAN M. MELLINKOFF, Nashville, Tenn. (Introduced by Elliot V. Newman).

A patient with unexplained thrombocytosis (platelet count 1 to 2 million per cmm. of blood) was noted to have a high serum potassium concentration (7.0 to 8.0 mEq. per liter) on routine determination. There was no clinical evidence of or explanation for the apparent hyperkalemia. The white cell count was normal. The serum potassium concentration could be correlated with the platelet concentration of the plasma or blood from which the serum was obtained. Serum from "platelet-free" plasma ($<$ 1000 platelets per cmm.) had a potassium concentration of 5.0 to 5.4 mEq. per liter, whereas "platelet-rich" plasma (2 to 3 million platelets per cmm.) yielded serum containing 8.0 to 9.5 mEq. per liter potassium. There was no evidence that the red cells were concerned in the results obtained. An appreciable amount of potassium was released from the platelets *immediately* after coagulation; it was not necessary for the clot to remain in contact with the serum for a prolonged period.

In three of six other patients with thrombocytosis this phenomenon was sufficiently marked so that serum from "platelet-rich" plasma contained from 1.3 to 2.1 mEq. per liter more of potassium than serum yielded by "platelet-free" plasma. There was no evidence that these platelets had a mass appreciably greater than that of normal platelets. In normal human subjects there was no consistent or significant influence of the platelets on the serum potassium concentration even when platelets were artificially concentrated *in vitro* to values approximating those of the patients with thrombocytosis. It is concluded that in some patients with thrombocytosis a false hyperkalemia may be attributed to the abnormal platelets.

The Production of Antibody by Partially Hepatectomized Rats. W. PAUL HAVENS, JR.,* MARY ELLEN SCHLOSSER, and JEAN KLATCHKO, Philadelphia, Pa.

The demonstration by Paschkis *et al.* that certain transmissible tumors had an increased rate of growth following implantation in partially hepatectomized rats suggested that an attempt be made to determine whether a specific stimulus associated with regeneration of liver might account for the enhanced production of antibody by many patients with hepatic cirrhosis. Four experiments, employing 192 adult male rats, were performed. Sheep erythrocytes were injected intravenously immediately after sub-total hepatectomy in 64 animals. Equally numbered groups of normal controls and laparotomized rats were also injected with the same antigen, and the titers of sheep-cell hemolysin were determined six days later. The geometric mean of the hemolysin titers (reciprocals) in the partially hepatectomized rats was 1310, compared with 570 and 520, respectively, in the normal controls and the laparotomized group. This difference is statistically significant, although not great enough (within the limits of accuracy of the test) to claim an increased potential in the partially hepatectomized rats.

There was no apparent relationship between changes in weight and the antibody produced. Approximately 40 per cent of the control and laparotomized groups gained during the experiment, while almost all the partially hepatectomized rats lost weight in significantly greater amounts than those which lost in the other two groups. Hypoalbuminemia and hyperglobulinemia are present for many days after partial hepatectomy or laparotomy. In partially hepatectomized rats, proliferation of the liver occurs, and after six days, when antibody titers were measured in these experiments, the mass of hepatic tissue was approximately its original magnitude. Under these conditions, with such great demands on available proteins, it is of interest that the partially hepatectomized rats made more antibody than their controls although the amounts made were insufficient to warrant the interpretation that any specific stimulating effect of regenerating hepatic cells was operative.

Vascular Adjustments after Phlebotomy in Polycythemic Subjects. HANS H. HECHT,* WALTER GAYLOR, and DAVID STEIN, Salt Lake City, Utah.

Hemodynamic adjustments of fifteen polycythemic patients were studied both before and after bleeding to normal packed red cell volumes. Four subjects had true erythremia, and eleven patients suffered from erythrocytosis with cor pulmonale secondary to emphysema. Microcytosis and reduced mean corpuscular hemoglobin concentration were common in the erythrocytosis group. None of the patients were in heart failure at the time of the study. Bleeding was carried out slowly over two to three weeks with a fall of packed red cell volume of 27 per cent (17–45). The usual methods for determination of respiratory and blood gas analysis, renal clearance, and plasma volume were employed. Pulmonary vascular pressures, right ventricular output and total pulmonary resistance were calculated, using the cardiac catheter.

All hemodynamic and renal data were normal in the erythremic subjects and remained so after bleeding. The absolute changes in the red cell mass were reflected in the plasma volume which rose from 31 ml. per kg. before, to 44 ml. per kg. after repeated bleeding.

In the patients with cor pulmonale no uniform response was obtained. Arterial oxygen content fell in all but one patient. Arterial oxygen saturation remained unchanged for the group with a mean of 66 per cent (53 per cent to 79 per cent) before, and 68 per cent (42 per cent to 84 per cent) after bleeding. Oxygen saturation rose moderately in four subjects, fell in two, and remained unchanged in five. Pulmonary artery pressures, elevated in all subjects with cor pulmonale, generally varied inversely with arterial oxygen saturation or remained unchanged. With the exception of two subjects in whom the onset of heart failure appeared to be precipitated by bleeding, no significant trends were observed in the other respiratory, circulatory, and renal functions studied.

Repeated bleeding may improve subjective symptoms of polycythemia and may lower blood viscosity. It has no predictable effect on the vascular alterations of cor pulmonale.

Lactate Metabolism in Cushing's Syndrome. DOROTHY H. HENNEMAN and JOHN P. BUNKER, Boston, Mass. (Introduced by Henry K. Beecher).

In an effort to clarify the disturbance in carbohydrate metabolism in Cushing's Syndrome, certain aspects of intermediary carbohydrate metabolism have been investigated. Plasma lactate was measured in twelve patients with Cushing's Syndrome and in one female patient with a masculinizing adrenal tumor with coincident features of Cushing's Syndrome. Fasting plasma lactate concentrations were significantly above normal. In three patients so studied the plasma lactate concentrations returned to normal two to three weeks post-operatively. Nine of these patients were studied during 70 to 180 minutes of ether anesthesia for adrenalectomy. All showed marked plasma lactate elevations during anesthesia which were significantly greater than the slight elevation in lactate of other patients under ether anesthesia, including two patients undergoing adrenalectomy for metastatic carcinoma. Pure d-lactate, according to Soffer's technique, was infused intravenously pre-operatively in seven patients with Cushing's Syndrome and all manifested a decreased lactate tolerance. Three dermatological patients treated with large doses of ACTH or cortisone for one to three months failed to show elevations in fasting plasma lactate concentrations or intolerance of d-lactate infusions. These findings suggest a decreased utilization of lactate in Cushing's Syndrome, perhaps due to disturbance in liver function. The failure to demonstrate similar changes after short term induced hyperadrenocorticism suggests that the duration rather than the severity of the disease is responsible for the lactate intolerance.

The Suppression of Urinary Calcium and Magnesium Excretion by Oral Sodium Phytate. PHILIP H. HENNEMAN and EVELYN L. CARROLL, Boston, Mass. (Introduced by Fuller Albright).

Our previous finding of increased calcium absorption as an explanation for the hypercalcuria of sarcoid patients emphasized the need for an agent which would antagonize vitamin D and decrease calcium absorption. The effect of sodium phytate on calcium, magnesium, sodium, potassium, phosphorus, and nitrogen metabolism has now been tested in four balance studies. Sixteen additional patients have been treated with this drug during the past 24 months.

Sodium phytate (inositol hexaphosphate) forms insoluble and apparently unabsorbable complexes with calcium and magnesium. In balance studies the daily oral administration of 9.0 grams caused increases in the daily fecal calcium ranging from 88 to 244 mg. and decreases in the urinary calcium ranging from 55 to 402 mg. (28 to 97 per cent). The same dose concomitantly caused increases in the fecal magnesium ranging from 17 to 88

mg. and decreases in the urinary magnesium ranging from 35 to 62 mg. per day. That the urinary calcium may be decreased to a greater extent than the fecal calcium is increased may be due to the absorption of inorganic phosphate released from sodium phytate by intestinal phytases.

The only adverse effect of the oral administration of 9 grams sodium phytate has been diarrhea which is usually mild and diminishes with time, and, more rarely, nausea and vomiting. It is of physiological interest that oral sodium phytate in the dose administered has only a small effect on calcium and magnesium balances, its main effect being to block absorption of dietary calcium and magnesium.

Sodium phytate may prove of specific therapeutic value in hypervitaminosis D, sarcoid and berylliosis with hypercalcuria, in certain types of renal lithiasis, and, possibly, in hemochromatosis. The latter prediction derives from the previously demonstrated *in vitro* removal of iron from solutions by sodium phytate.

The Effects of Parathyroid Extract on Renal Function in Man. HOWARD H. HIATT and DAVID D. THOMPSON, Bethesda, Md. (Introduced by Ephraim Shorr).

The mechanism of the increase in urinary phosphate which follows the administration of parathyroid extract has been a subject of controversy. We have studied the acute effects on renal function of parathyroid extract and its action after prolonged administration.

In 28 studies on six normal and two hypoparathyroid subjects renal reabsorption of phosphate was calculated at endogenous and elevated serum phosphate levels. Inulin clearances were determined in all studies. The infusion of buffered sodium phosphate permitted calculation of maximal tubular reabsorption of phosphate (Tm P).

Intravenous administration of 200 to 1000 USP units of parathyroid extract (Lilly) produced no immediate measurable change in TM P in five normal subjects in 11 studies, and a 40 per cent depression in Tm in two hypoparathyroid individuals. A second preparation of extract given to one of these hypoparathyroid subjects did not alter phosphate Tm. In all studies, a prompt rise in renal blood flow and urinary phosphate was observed. In the studies in which phosphate Tm was unchanged, the increased phosphate excretion was attributable to the rise in filtered phosphate secondary to an increased glomerular filtration rate.

Administration of 1600 to 4000 units of extract over 48 to 96 hours produced a 20 to 60 per cent fall in phosphate Tm in all normal and hypoparathyroid subjects. The depression in Tm occurred after prolonged administration of all preparations of extract employed. All preparations produced hypercalcemia.

Our studies support the following conclusions:

1. Renal tubular reabsorption of phosphate is depressed by parathyroid extract, although an effect is demonstrable in normal subjects only after prolonged administration.

2. The increased urinary phosphate observed in normal individuals following acute administration of extract is generally attributable to a rise in filtered phosphate.

3. Preparations of extract vary in their capacity to depress phosphate Tm acutely in hypoparathyroid subjects.

Observations of Magnesium Metabolism in Man. A. G. HILLS, D. W. PARSONS, O. ROSENTHAL, and G. D. WEBSTER, JR., Philadelphia, Pa. (Introduced by William A. Jeffers).

Evidence that renal magnesium excretion is influenced by renal sodium chloride excretion was obtained in humans. Seven subjects, each kept on a constant diet throughout, were observed during a control period followed by a 3- or 4-day experimental period in which the only change of regimen was withdrawal of a sizable supplement of enteric-coated sodium chloride. Twenty-four hour urinary excretion of sodium chloride and magnesium decreased during the experimental period in all subjects, the mean change of urinary magnesium being -21.8 ± 3.7 per cent ($P < .01$). Quantitatively similar results (urinary magnesium decrease 28 ± 6.6 per cent, $P < .05$) were obtained in four adrenal-deficient patients in whom maintenance steroids were withdrawn together with the salt supplement. The changes reflected renal tubular activity since the ratio magnesium clearance: glomerular filtration rate fell.

In four of five subjects studied, fecal as well as urinary magnesium decreased. Definite rise of serum magnesium concentration was observed only in adrenal-deficient subjects developing evidence of adrenal insufficiency. Net magnesium retention of the indicated magnitude without change of extracellular magnesium concentration would suggest homeostatic transfers of magnesium out of extracellular fluid in subjects with adequate adrenal function.

Six-day adrenal stimulation with ACTH significantly decreased urinary excretion of magnesium, sodium, and chloride in another subject, and cortisone had similar effects in a totally adrenalectomized hypertensive, sodium chloride intake being constant in both patients. Hormone withdrawal exerted opposite effects.

The data indicate that adrenal cortical hormones influence urinary magnesium excretion only insofar as they affect urinary sodium and chloride excretion. Urinary potassium excretion appears unrelated to urinary magnesium. In experiments effecting change of 24-hour urinary excretion of sodium *or* chloride ion independently, urinary magnesium excretion paralleled urinary chloride, but urinary sodium and magnesium excretion varied in opposite directions.

Abnormalities of Energy Metabolism in Congestive Heart Failure and Their Quantitative Relationship to Circulatory Changes. WILLIAM E. HUCKABEE, Boston, Mass. (Introduced by Chester S. Keefer).

Measurements of tissue anaerobic metabolism were derived from changes in relationships between lactate and pyruvate net increment rates. Balances between aerobic and anaerobic energy turnover constitute measures of

"adequacy" of oxygen supply for the support of intracellular oxidative processes, independent of blood flow or tissue demand taken separately. In 11 normal subjects and 18 patients with heart failure this method was applied to the whole body through analysis of arterial blood, and integrated with cardiac catheterization data.

Cardiacs relied on chemically anaerobic mechanisms to meet cellular energy requirements much more than normal subjects, with no overlapping of data from the two groups. In normals 92 to 98 per cent of tissue energy demands in mild exercise were met by circulatory response. Calculations showed that patients with diseased hearts increased cardiac output by only 30 to 60 per cent as much as called for by metabolic needs; and only 50 to 80 per cent of oxygen required by the tissues was delivered, despite increased blood oxygen extraction; the remainder of total energy expenditure was supplied by peripheral tissue anaerobic mechanisms (61 to 185 ml. O_2-equivalents per min.). Thus O_2 consumption gave a grossly erroneous estimate of exercise severity in patients.

Results of this estimate of "circulatory adequacy" were not predictable from observed changes in O_2 consumption, $(A\text{-}V)O_2$ or cardiac pressures, whether these were greatly altered or entirely normal, as in six cases of Class I heart disease and nine of post-valvulotomy mitral stenosis, which showed metabolic abnormalities of characteristic types.

Conclusions: 1) Insufficiency of blood supply to the body is a possible basic mechanism in the syndrome of heart failure, regardless of cardiac output or even of venous blood oxygen.

2) Major compensatory responses to circulatory insufficiency include not just increase in vascular pressures and blood oxygen extraction, but also significant utilization of energy sources other than oxygen (anaerobic glycolysis) to maintain life processes, an abnormality extremely sensitive to cardiac impairment.

Method for the Prevention of Ventricular Fibrillation during Hypothermia. CHARLES A. HUFNAGEL, PIERRE RABIL, and JOHN C. ROSE, Washington, D. C. (Introduced by Laurence H. Kyle).

The use of hypothermia for open heart procedures offers direct correction of complicated defects, but the high rate of ventricular fibrillation during ventriculotomy below 26° C. has been a deterrent to its application. This study attempts to compare the value of intravenous Prostigmin,® S-A nodal block with Xylocaine, and Prostigmin® plus S-A nodal block in preventing ventricular fibrillation in dogs with temperatures below 26° C.

The animals were divided into four groups. In group I a standard procedure was established. The rectal temperature was lowered to between 24 and 26° C., both venae cavae were occluded for 15 minutes, and a right ventriculotomy was performed. Under these conditions ventricular fibrillation occurred in at least 90 per cent of the animals. In group II an identical procedure was carried out except that at 30° C., .025 mg. Prostigmin® per pound of body weight was given intravenously. This was repeated with each degree of temperature fall thereafter until 26° C. In this group ventricular fibrillation was infrequent, and when it occurred, defibrillation was easily accomplished. In group III, immediately following opening of the chest, the S-A node was injected with 5 cc. of 1 per cent Xylocaine. Fibrillation occurred with approximately the same frequency as in group II. In group IV Prostigmin® was given as in group II, and following thoracotomy the S-A node was blocked with Xylocaine as in group III. Caval occlusion and ventriculotomy were carried out as in all groups. Ventricular fibrillation occurred in only one animal in this group.

Group IV animals showed a high rate of permanent recovery as well as protection against ventricular fibrillation. The combination of intravenous Prostigmin® in large doses and infiltration of the S-A node with Xylocaine appeared to provide better protection against ventricular fibrillation than either method alone.

The Origin of the Early Systolic Click of the Pulmonary Artery. HERBERT N. HULTGREN and JOHN J. KELLY, JR., San Francisco, Calif. (Introduced by Arthur L. Bloomfield).

An early systolic sound, frequently clicking in character and closely following the apical first sound, is occasionally heard at the pulmonic area in patients with pulmonary hypertension. During the past four years we have studied 45 cases exhibiting the early systolic click of the pulmonary artery with particular reference to its mode of production. The following observations have been made:

1. The first sound-click time is related, as pointed out by Leatham, to the duration of isometric contraction of the right ventricle. Isometric contraction times, measured by means of intracardiac pressure tracings and electrokymography in 100 patients with varying degrees of pulmonary hypertension, were found to be prolonged in proportion to the elevation of pulmonary artery diastolic pressure.

2. Three patients have been studied in whom a shortening of the P-R interval was associated with a delay and an increased intensity of the first sound but no change in position or intensity of the pulmonary sound.

3. Two patients without systolic murmurs exhibited loud Graham Steell murmurs which continued through the first sound and ended an instant before the pulmonary click.

4. Phonocardiograms recorded from the exposed heart during surgery revealed the click to be most intense over the main pulmonary artery and faint over the right ventricle or distal pulmonary artery. An abrupt checking of systolic expansion was palpable at the pulmonary valve ring but not over the artery.

5. Unsuccessful attempts have been made to reproduce the sound by suddenly tensing in a perfusion apparatus pulmonary arteries obtained at autopsy.

These observations indicate that the early systolic click of the pulmonary artery is not due to A-V valve closure

but that it is probably produced by sudden early systolic tensing of the fibrous pulmonary valve ring. Transmission to the chest wall is facilitated by pulmonary artery dilatation.

Sodium, Potassium, and Magnesium Balance during Recovery from Congestive Heart Failure Due to Cor Pulmonale. LLOYD T. ISERI and IVAN J. MADER, Detroit, Mich. (Introduced by William O. Maddock).

Metabolic studies conducted during recovery from heart failure due to low and high (beri-beri) output heart disease have previously shown uptake of cellular K and Na, and frequent loss of water. Calculations have also indicated inactivation of cellular base during this period.

A complete metabolic balance study of Na, K, Cl, Mg, N, and H_2O, using a constant low sodium diet of known analyzed composition, was undertaken during recovery in one case of cor pulmonale and in two cases of low-output failure. Measurement of cardiac, pulmonary, and renal hemodynamics, obtained by cardiac catheterization technique before and after recovery, established the diagnosis in the case of cor pulmonale.

Metabolic studies in all three patients, including the one patient with cor pulmonale, showed a marked loss of extracellular water, Na, and Cl, a slight gain of intracellular water, a marked gain of intracellular sodium, and a moderate gain of intracellular potassium. Two patients showed a gain of intracellular magnesium, but one patient showed a loss. All three patients showed a marked inactivation of cellular base.

These studies indicate that intracellular changes during recovery from congestive heart failure due to cor pulmonale are similar to those found in beri-beri and in low-output heart disease. The hypothesis that increased osmolarity of intracellular base following cardiac and circulatory insufficiency may be responsible for the train of events leading to congestive cardiac edema appears to apply in cor pulmonale as well as in other heart diseases.

Uridine Compounds: Their Role in Hormonal Glucuronide Formation. KURT J. ISSELBACHER and JULIUS AXELROD, Bethesda, Md. (Introduced by Seymour S. Kety).

The significance of uridine compounds in biologic reactions has become increasingly apparent in the past few years on the basis of the work of Leloir, Dutton, Storey, and Kalckar. These studies have included the isolation from mammalian tissues of uridine diphosphate (UDP) derivatives—including UDP-glucose, UDP-glucuronic acid, and UDP-N-acetyl glucosamine. The exact functions of these compounds in biologic systems remains to be elucidated.

It is known that the metabolites of adrenal, thyroid, and sex hormones are excreted in significant amounts as glucuronide conjugates, but the mechanism of their formation has remained obscure. Since free glucuronic acid or its lactone do not function as direct precursors of these glucuronides, we have investigated the possible role of UDP-glucuronic acid in hormonal glucuronide synthesis.

When tetrahydrocortisone is incubated with a cell-free preparation of mammalian liver, the addition of UDP-glucuronic acid results in the formation of tetrahydrocortisone glucuronide and the liberation of UDP. The enzyme catalyzing this transfer reaction is localized in the microsomal fraction of the liver cell and has been partially purified. In a similar manner we have obtained glucuronide formation of other hormones such as tetrahydro-hydrocortisone and L-thyroxine, and of non-hormonal substances such as phenolphthalein and morphine.

It would appear that UDP-glucuronic acid is an "active" form of glucuronic acid and an essential cofactor for the synthesis of glucuronides by the mammalian organism.

A Hemorrhagic State Following Multiple Whole Blood Transfusions. DUDLEY P. JACKSON and JULIUS R. KREVANS, Baltimore, Md. (Introduced by C. Lockard Conley).

A serious hemorrhagic disorder has occasionally followed the administration of multiple blood transfusions. To determine the cause, 29 adult patients and 5 newborn infants who received one or more compatible whole blood transfusions were studied. Sixteen adults who received more than 5,000 ml. of whole blood within 48 hours developed thrombocytopenia following the transfusions, and 12 exhibited clinical evidences of abnormal bleeding. In 14 cases, platelets were reduced to 60,000 per cmm. or less. Other studies of hemostatic function were performed in 7 patients who developed a hemorrhagic state. Fibrinogenopenia was encountered in only one instance, and evidence of increased fibrinolytic activity was not found. Some of the 13 adult patients who received less than 5,000 ml. of whole blood within 48 hours developed mild thrombocytopenia, but none had abnormal bleeding. In five consecutive cases of exchange transfusion for erythroblastosis fetalis, all of the infants developed severe thrombocytopenia, and 2 displayed clinical evidences of abnormal bleeding. The thrombocytopenia that was observed in these patients was related to the amount of whole blood transfused and to the rate of infusion. Its development was not prevented by the use of non-wettable blood bank equipment nor by the use of freshly drawn blood. Direct transfusion, using multiple silicone-coated syringes, failed to prevent the occurrence of thrombocytopenia in one case. Thrombocytopenia developed even though splenectomy was performed in one instance. The platelet depression persisted for three to five days following transfusion in most cases.

Thrombocytopenia has been produced in dogs following the administration of large amounts of compatible blood from donor dogs. Auto-transfusion of comparable quantities of whole blood administered by the indirect technique did not produce thrombocytopenia.

Alterations of the Plasma Protein Mosaic in Pneumococcal Infections in Rabbits. RALPH F. JACOX * and ALEXANDRA FELDMAHN, Rochester, N. Y.

A type 1 pneumococcal "dermal pneumonia" was produced in adult male rabbits and the sequence of plasma protein alterations was determined at daily intervals utilizing a cationic detergent fractionation method (Jacox, R. F., J. Clin. Invest., 32: 661, 1953; Jacox, R. F., J. Lab. & Clin. Med., 44: 885, 1954). Two days after the injection of pneumococci the rabbits developed a striking rise of plasma fibrinogen and alpha globulins. These fractions remained at high concentrations for eight days and by the tenth day slowly returned toward control levels. Between the second and tenth days of infection, plasma albumin was depressed and then rose slowly toward normal levels during the second and third week. The plasma beta-gamma fraction was decreased at the height of infection and then returned to normal or slightly elevated values during convalescence.

Five of thirteen rabbits with untreated pneumococcal infections developed a severe illness as judged by the intensity of pneumococcal bacteremia. Five others had a milder illness with a transitory and mild bacteremia. While each of these groups showed similar alterations in the plasma protein mosaic, those severely ill had significantly less elevation of the plasma alpha globulins within the first 24 hours after onset of infection. The severely bacteremic animals thereafter, developed a significantly greater rise of plasma alpha globulins and fibrinogen and a greater fall of plasma albumin than occurred in the mildly bacteremic group. Administration of penicillin 48 hours after the injection of type 1 pneumococci in another group of rabbits, partially inhibited the development of the plasma protein alterations observed in the untreated series.

These experiments indicate that the stimulus for synthesis of fibrinogen and alpha globulins and for depression of the albumin level is directly related to the intensity of stimulus produced by the infecting agent. The data also suggest that an *early* failure of the rabbit to synthesize plasma alpha globulins may predispose the animal to the development of a more severe bacteremia.

Erythrocyte Destruction and Hemoglobin Turnover in Severe Acquired Hemolytic Anemia Studied with N^{15}-Glycine. G. WATSON JAMES, III * and LYNN D. ABBOTT, JR., Richmond, Va.

The response of the bone marrow to a continuing demand for erythropoiesis and hemoglobin synthesis has been studied in two elderly patients with severe acute acquired hemolytic anemia.

A 75-year-old man with 6.3 gm. per cent hemoglobin, 1.44 million red cells and 52 per cent reticulocytes; and a 70-year-old woman with 5.6 gm. per cent hemoglobin, 1.39 million red cells, and 60 per cent reticulocytes were in a stabilized hematologic state as evidenced by serial hemograms during the period of study. The nitrogen of the heme of their erythrocytes was labeled by the oral feeding of *one* gram of N^{15}-glycine (31 atom per cent excess), thus affording a means of following the disappearance of red cells from the individual's own circulation. The marked reticulocytosis was evidence of the rapid erythropoiesis. In the absence of a hemolytic process erythrocytes formed during an accelerated erythropoiesis with reticulocytosis live a normal life span (Clin. Res. Proc., 1953, 1, 64).

Approximately five times as much heavy nitrogen (N^{15}) was incorporated into the heme of these subjects when compared with a normal male given the same amount of glycine. The rapid rate and pattern of disappearance of the isotope from the circulating heme could be interpreted as due to random destruction of erythrocytes with an extremely short half life of about five days, indicating a mean life-time for the major portion of the red cells of about 7 days, and a turnover rate for hemoglobin of about 15 per cent per day. From total circulating hemoglobin by T-1824 technique it was calculated this represented hemoglobin synthesis and destruction of about 36 gm. per day. This may represent a close approximation to the limiting value for hemoglobin synthesis, and is further evidence of the remarkable functional capacity of the bone marrow.

Mechanisms of the Defect in Neuromuscular Function in Familial Periodic Paralysis. RICHARD J. JOHNS, ÅKE LILJESTRAND, DAVID GROB,* and A. MCGEHEE HARVEY,* Baltimore, Md.

A patient with familial periodic paralysis was studied during and after attacks of paralysis. Both spontaneous attacks and attacks precipitated by glucose administration were studied. Muscle action potentials in response to supramaximal ulnar nerve stimuli were recorded from the abductor of the fifth finger or adductor of the thumb. The isometric twitch tension of the adductor of the thumb was also recorded. The electrocardiogram and serum potassium levels were followed concurrently.

The normal steps in neuromuscular transmission are: 1) Liberation of acetylcholine by the nerve ending in response to a nerve impulse; 2) Depolarization of the motor end-plate by the acetylcholine, producing the end-plate potential; 3) initiation of the muscle action potential by the end-plate potential; 4) Propagation of the muscle action potential along the muscle fiber giving rise to muscular contraction. The sensitivity of the motor end-plates to depolarization by acetylcholine was tested by the intra-arterial injection of acetylcholine. This test demonstrated that during paralysis the end-plates were highly resistant to depolarization by acetylcholine. Other evidence showed that this resistance to depolarization was not due to the presence of a competitive (curare-like) block at the neuromuscular junction.

During paralysis the electromyogram showed abnormalities of depolarization and repolarization of the muscle fibers. Records of action potentials obtained from successive points along the muscle indicated interference with the normal propagation of depolarization along the muscle fibers. The electrocardiogram showed only

marked abnormalities of repolarization. With recovery from the paralysis, either spontaneously or due to administration of potassium chloride, all the abnormalities disappeared.

Thus, there appear to be at least two causes contributing to the paralysis seen in this disease. First, there is partial neuromuscular block due to resistance of the motor end-plate to depolarization by acetylcholine. Second, there are abnormalities of propagation of the muscle action potential.

Correlations of Serial Renal Biopsies and Other Data in Patients with the Nephrotic Syndrome. Robert M. Kark,* Robert C. Muehrcke, Conrad L. Pirani, and Victor E. Pollak, Chicago, Ill.

The progression of pathologic changes in kidneys of patients ill with the nephrotic syndrome was studied by serial renal biopsies, and compared with biopsies from non-nephrotic controls. Histologic data were correlated with changing clinical status, clinical laboratory data, and gross and discrete renal function tests in a continuing study of the pathophysiology and natural history of different renal diseases.

Forty adults who developed the nephrotic syndrome (criteria of Leiter) were biopsied. Serial biopsies (up to four in 6 patients) were done in 14. Diagnoses included: glomerulonephritis (14); diabetes mellitus (11); lupus erythematosus disseminatus (7); renal vein thrombosis (2); possible lipoid nephrosis (2); primary renal amyloidosis (1); and undiagnosed (3). In glomerulonephritis, the earliest detectable lesions were interstitial edema and tubular cellular degeneration. Subsequent biopsies showed development of glomerular changes. Azotemia was associated with interstitial edema and tubular changes, disappeared as these improved, and reappeared when glomeruli became damaged. Changes seen during massive diuresis following corticotropin therapy will be demonstrated.

There was no correlation between the degree of proteinuria and extent of glomerular involvement. In fact, proteinuria was inversely related to the glomerular damage. The concept that protein is filtered by glomeruli and reabsorbed by tubules was supported by finding proteinaceous material in Bowman's space around normal glomeruli in biopsies from non-nephrotic patients without proteinuria. Usually there was good correlation between renal function tests and structural changes. These findings, together with previous data, relate the pathophysiology of the nephrotic syndrome to diffuse renal interstitial edema.

The Occurrence of Acid Mucopolysaccharides in Human Leukocytes. Grace P. Kerby,* Durham, N. C.

The ground substance of connective tissue has as its major component high polymeric compounds called acid mucopolysaccharides, composed of repeating units of disaccharides of hexuronic acid and hexosamine. In most instances, the hexosamine is acetylated, the disaccharide esterified with sulfuric acid, and the compounds loosely bound to protein. The compounds exhibit metachromatic staining. The reaction of ground substance to any form of injury plays an important role both in the type of response to and the recovery from injury. Regulatory mechanisms are unknown. Because of the important role played by the leukocyte in inflammatory reactions of all kinds, and because of the failure of certain types of tissue reaction to injury (*i.e.*, the Shwartzman phenomenon) to occur in the absence of leukocytes, the acid mucopolysaccharides in human leukocytes are worthy of study.

Human leukocytes yield a non-dialyzable, metachromatic-staining material which contains hexuronic acid and hexosamine. Expressed in terms of hexuronic acid content, leukocytes from males yielded 1180 ± 52 (S.E.) gamma, females 900 ± 48 gamma of the material per 10^9 leukocytes (said by Rossiter to equal 4 gm. wet weight of leukocytes). The difference between the yield from the leukocytes of males and of females was slight but highly significant ($p = < 0.01$). Chromatographically the major component of the material from human leukocytes had the same R_f value as did the major component of both a similar material isolated from human urine and a commercial brand of chondroitin sulfate. The material from leukocytes was homogeneous in this R_f range and in this respect was unlike both the human urine material and the commercial chondroitin sulfate.

The Relation of Steroid Structure to the In Vivo Reactivation of Influenza B Virus. Edwin D. Kilbourne, New Orleans, La. (Introduced by C. Thorpe Ray).

Evidence has been presented that injection of chick embryos with cortisone results in reactivation to a state of infectivity of thermally inactivated influenza B virus. This effect is mediated by the action of cortisone on the host cell of the cell-virus complex, and not by a direct effect upon the virus. Investigation of the specificity of this effect has revealed that it is not confined to cortisone but may be achieved also by the administration of other 11-oxygenated corticosteroids (Compounds A, B, F) and by such biologically diverse C-21 steroids as progesterone, desoxycorticosterone (DOC), and Reichstein's Compound S. However, specificity of the reaction is implicit in the failure of 11αhydrocortisone (11 epi-F) and 11αhydroxy progesterone to induce reactivation or increase of virus. Thus, abnormal orientation of the C-11 hydroxyl group is equated with inactivity of compounds whose stereoisomers (11-β-hydroxy-) are highly active in effecting viral reactivation.

Despite this evidence of the importance of oxygenation at C-11, the activity of compounds desoxy- at that position (DOC, progesterone) has been repeatedly demonstrated. These apparently divergent findings may be reconciled by an hypothesis that β-hydroxylation at the 11th carbon atom may be effected by the chick embryo. An endogenous 11-β-hydroxylase might thus convert DOC and progesterone to 11-oxy-steroids of the gluco-

corticoid type, but would be ineffectual in the conversion of inactive compounds already hydroxylated in the α orientation at C-11.

Production of Renal Ischemia and Proteinuria in Man With Intravenous L-norepinephrine and epinephrine. S. EDWARD KING and DAVID S. BALDWIN, New York, N. Y. (Introduced by Joseph Post).

Clinical manifestations of vasomotor instability and pronounced systemic reactions to postural change and other environmental influences with demonstrable renal ischemia are frequently observed in subjects with intermittent proteinuria. In this study renal ischemia and proteinuria have been induced in man through the intravenous administration of l-norepinephrine and epinephrine (USP).

Observations of glomerular filtration rate (GFR), renal plasma flow (RPF), urine volume and protein excretion were made under basal hydropenic conditions in 10 subjects with l-norepinephrine and 4 with epinephrine (USP), administered intravenously at constant infusion rates varying from 15 to 45 μg. per min.

Proteinuria was induced by l-norepinephrine in 8 out of 10 subjects and by epinephrine in all 4. Small doses of l-norepinephrine were employed in the 2 subjects failing to exhibit proteinuria. Proteinuria persisted for periods up to 50 minutes following discontinuation of these amines while renal functions were returning to control levels. No difference of response was observed between normal subjects and those with known intermittent proteinuria.

Marked reduction in RPF occurred particularly with larger doses of l-norepinephrine (mean 57 per cent) and with epinephrine (mean 51 per cent). The GFR was reduced following larger doses of l-norepinephrine (mean 38 per cent) and after epinephrine (mean 32 per cent).

Increased renal vascular resistance was preponderantly afferent with l-norepinephrine, and afferent and venular with epinephrine.

In 5 normal subjects, the maximal tubular reabsorptive rate for glucose (TmG) was unaffected by l-norepinephrine infused at a rate of 30 μg. per min. This suggests that the renal ischemia induced was diffuse.

Proteinuria accompanied by renal ischemia was induced in man by intravenous l-norepinephrine and epinephrine. These data suggest that the adrenal medullary hormones play a role in production of transient proteinuria in various stressful conditions.

Steroid Hormonal Metabolites of Meconium. RALPH A. KINSELLA, JR. and FAITH ELLEN FRANCIS, St. Louis, Mo. (Introduced by Ralph A. Kinsella).

Recently, we reported the isolation and crystallization of estriol from human meconium, thus establishing excretion by human intestinal route of this metabolite. Further studies have indicated the excretion in meconium of 17-ketosteroids. However, no evidence for the excretion of significant amounts of water soluble ketonic corticoids has been found by Zaffaroni's paper chromatographic analysis for substances reducing ammoniacal silver or by analysis with Porter and Silber's reagent for 17-hydroxy alpha-ketols. The extraction procedure consisted of preparation of a butanol extract, evaporation of the solvent, suspension of the residue in water, extraction of the aqueous suspension with ligroin and with ethyl ether. After incubation with bacterial betaglucuronidase, extraction with ethyl ether was carried out. Sulfate conjugates of the estrogens were hydrolyzed with phenol sulfatase and the estrogens were removed by extraction with ether. Hydrolysis of the sulfate conjugates of the 17-ketosteroids was carried out by continuously extracting with ether for 24 hours at pH 0.7. The extract of each hydrolysate was subjected to Girard's separation procedure. A benzene solution of the ketonic fraction was repeatedly extracted with water to remove corticoids and then with alkali to remove phenols. Ten mg. of 17-ketosteroids per kilogram of meconium were obtained after hydrolysis with beta-glucuronidase and 2 mg. per kilogram after continuous extraction at pH 0.7. Preliminary paper chromatographic analysis of the neutral fractions indicated that the bulk of the 17-ketosteroids was relatively more polar than testosterone, with the exception of the principal component of the sulfate fraction which moved about twice as fast.

Effect of Mild Steady State Exercise on Cerebral and General Hemodynamics of Normal Untrained Subjects. JEROME KLEINERMAN and SALVATORE M. SANCETTA, Cleveland, O. (Introduced by Roy W. Scott).

The effect of mild exercise (120 to 135 foot pounds per minute) on the general and cerebral hemodynamics has been studied in 10 normal untrained subjects in the prone position, employing a variable resistance bicycle ergometer. The amount of work which would insure a steady state within 10 minutes and maintain it for an additional 20 minutes as gauged by oxygen consumption was predetermined the day before the study. Cerebral blood flow (CBF) was determined by the nitrous oxide method of Kety and Schmidt, the cardiac output by the direct Fick principle and pressures transduced *via* Statham strain gauges.

There were significant increases in mean minute ventilation, oxygen consumption per M^2 (123 cc. to 221 cc., $p = < .001$) and total AV difference, with significant decreases in pulmonary vascular (242 to 190 c.g.s., $p = < .05 - > .01$) and total peripheral resistance (1346 c.g.s. to 1087 c.g.s., $p = < 0.5 - > .01$). Mean minute respiratory rate increased from 15.6 to 20.3 per min. and heart rate from 81 to 87 beats per min. Mean pulmonary and brachial artery pressures showed no significant changes.

The CBF fell from a mean of 56 cc. per 100 gm. per min. at rest to 49 cc. in exercise ($p = < .05 - > .01$). The cerebrovascular resistance concomitantly increased from 1.8 to 2.0 units ($p = < .05 - > .01$). The cerebral oxygen consumption did not change significantly during exercise (3.2 cc. per 100 gm. per min. at rest, 3.1 cc. per 100 gm. per min. in exercise). The respiratory quotient

of the brain similarly was unchanged in exercise (.91 at rest, and .92 in exercise). The decreased CBF is probably dependent on the fall in arterial pCO_2 during exercise. At rest the $ApCO_2$ was 43 mm. Hg as compared to 41 mm. Hg during exercise ($p = < .05 - > .01$). From these observations it appears that the brain does not participate in the general body decrease in peripheral resistance during mild exercise.

The Action of Iodoacetate on the Electrical and Mechanical Activities of the Isolated Perfused Frog Heart. MORRIS KLEINFELD, EDWARD STEIN, and JOHN MAGIN, New York, N. Y. (Introduced by Charles E. Kossmann).

Microelectrodes were inserted into the interior of single ventricular fibers of the isolated perfused frog heart and the effects of 5×10^{-4} M iodoacetate on the intracellular potential, the indirect electrocardiogram and the direct cardiac output were recorded. Prominent changes in both the electrical and mechanical properties were observed. The most consistent, early change was a shortening of the action potential duration (AP_4) which was progressive. Coincident with this a shortened S-T interval and abnormal T wave were observed in the indirect electrocardiogram. Of lesser frequency was a prolongation of the P-R interval. A decreased height in the membrane action potential (MAP) with impaired depolarization occurred relatively late. The change in mechanical activity was represented by a progressive decrease in cardiac output, stroke volume and heart rate. Auricular activity persisted for sometime after ventricular arrest. The alterations in mechanical activity were usually paralleled by changes in the repolarization phase of the action potential (shortened AP_4) of the ventricular fiber. The addition of substrates such as pyruvate (0.0055 M) and acetate (0.0055 M) to the IAA treated heart produced only a partial recovery and delay of ultimate deterioration of cardiac activity. The result was similar when adenosine triphosphate (1.0×10^{-4} M) was added.

The results obtained confirm the previous observations of the effect of IAA on the mechanical activity of the heart but are at variance with the findings and conclusions with regard to the electrical activity. The recording of several parameters of the electrical potentials in contrast to only the height of the surface action potential as measured by Gardner *et al.*, revealed an early effect on the electrical properties which mirrored the changes in cardiac output.

The inability of added pyruvate, acetate, and ATP to restore the cardiac activity suggests that under the conditions of these experiments IAA has other actions in addition to that of a specific inhibitor of triose—phosphate dehydrogenase. In line with Hodgkin's theory of membrane permeability it is postulated that the enhanced repolarization phase is associated with an increased permeability of the cellular membrane to K. Observations of Love *et al.* of an increased outflow of K and net gain of Na in human erythrocytes following exposure to IAA lends support to this postulate.

The Protective Effect of Hypercalcemia in the Hyperkalemic State. MILTON J. KRAININ, CHARLES L. WHISNANT, and JOSEPH S. WILSON, Atlanta, Ga. (Introduced by Arthur J. Merrill).

Renewed clinical interest has been shown in the possible protective action of calcium against the toxic effects of hyperkalemia. Previous experimental data appear to show that when serum calcium levels are high there may be marked increases in potassium tolerance. Improvement in the electrocardiographic abnormalities of hyperkalemia shortly after the intravenous injection of calcium gluconate has been demonstrated clinically.

The procedure in this study was to infuse intravenously a solution of potassium chloride into two groups of normal anesthetized dogs. One of the groups received calcium gluconate simultaneously. Plasma potassium and calcium as well as other electrolytes and electrocardiograms were followed at frequent intervals in both groups until death. Results representing 20 dogs in the control group and 20 dogs receiving calcium gluconate showed that the average venous plasma potassium level shortly prior to death in the control group was 11.2 ± 1 mEq. per L. and that in the group receiving calcium gluconate was 12.2 ± 1.5 mEq. per L. ($P < .02$). At the time these samples were drawn, calcium levels in the group receiving calcium gluconate averaged 19.3 mg. per 100 ml. Those not receiving calcium required an average of 143 mg. KCl per lb. to produce death while those receiving calcium required 173 mg. KCl per lb. This difference was not statistically significant ($P > .6$). Similar results were recorded in a group of ten dogs in whom both ureters had been ligated three days before the above procedure was carried out. Five dogs acted as controls and five were given calcium.

It is concluded that increased serum calcium levels appear to offer slight protection against hyperkalemia under the conditions of the experiments.

The Effect of Thyroid Disease on the Metabolism of Calcium[45] in Man. STEPHEN M. KRANE, GORDON L. BROWNELL, and JOHN B. STANBURY,* Boston, Mass.

Increased calcium excretion resulting in negative calcium balance has been observed in patients with hyperthyroidism; decreased calcium excretion has been observed in patients with myxedema. This study was designed in an attempt to define these changes using radioactive calcium (Ca^{45}).

Ca^{45} (5 microcuries) was administered intravenously to four patients with hyperthyroidism, two with myxedema and one normal, all of whom were on constant low calcium diets. Specific activities (per cent administered dose Ca^{45} per gram stable calcium) were determined on serum, urine, and feces. Semilogarithmic plot of urine and serum specific activities versus time suggests curves composed of three decreasing exponential functions. Dif-

ferences among patients were most marked in the third slope which appeared by the second day. This slope ranged from 24.3 to 28.8 per cent per day in the hyperthyroid patients and was 8.1 per cent per day in the normal patient. The values were 6.3 and 7.2 per cent per day in the myxedema patients and rose to 15.0 and 17.4 per cent per day 6 months after treatment with thyroid.

At an arbitrary time of nine days following the administration of Ca^{45}, a calcium space was calculated:

$$\frac{\text{(\% administered dose remaining after 9 days)}}{\text{urine specific activity day 9}}$$

This value ranged from 47.3 to 247.7 grams in the hyperthyroid patients and 10.8 grams in the normal patient. In the myxedema patients it was 5.0 and 5.4 grams, before treatment, and 13.0 and 18.2 grams after treatment.

Since the marked fall in serum and urine specific activities in patients with hyperthyroidism is not accounted for by increased excretion rate of Ca^{45}, it appears that more Ca^{45} enters the body calcium spaces, more rapidly than normal. The reverse is found in patients with myxedema.

The Effect of Intravenous Heparin on Lipemia-Induced Angina Pectoris in Patients with Coronary Artery Disease. PETER T. KUO and CLAUDE R. JOYNER, JR., Philadelphia, Pa. (Introduced by Charles C. Wolferth).

Lipemia was induced in 20 patients with coronary artery disease and angina pectoris by a standard fat meal. Electrocardiogram; ballistocardiogram; pneumogram; plasma lactescence; serum cholesterol, phospholipid, and fatty acid; and serum lipoprotein (paper electrophoresis) were studied before, at intervals after the test meal, and 15 minutes after intravenous administration of heparin.

One to four attacks of angina pectoris were regularly precipitated on different occasions, in each of eight patients in this series by the ingestion of the fatty meal. These patients developed a total of 19 attacks of anginal pain three to five hours after meal, when their postprandial lipemia was near its peak level as indicated by plasma lactescence and serum lipid determinations. Electrocardiographic changes of acute myocardial ischemia, deterioration of the ballistic complexes, and an increase in the rate and depth of the respiration were observed during each attack.

Thirteen anginal attacks ceased five to seven minutes after the intravenous administration of 5 to 25 mg. of heparin irrespective of the previous duration of the anginal pain. This was accompanied by reversion of the electrocardiogram, ballistocardiogram, and pneumogram to their respective controlled levels. More rapid relief was observed on five occasions with nitroglycerine. One patient developed prolonged substernal distress four and a half hours after the fatty meal, with persistent electrocardiographic and ballistocardiographic changes. He did not respond to heparin or nitroglycerine therapy. Two anginal attacks were not influenced obiectively or subjectively by saline injections, but were subsequently relieved by heparin.

In one patient, the anginal pain was initially relieved by nitroglycerine, but he experienced a recurrence of symptoms 35 minutes later. This was controlled by 5 mg. of heparin intravenously.

Heparin alters plasma lactescence and serum lipoprotein migration on filter paper. The relation of these to the relief of angina remains to be determined.

The Metabolism of Intravenously Infused C-14 Labelled Cholesterol in Man Before and After the Induction of Myxedema. GEORGE S. KURLAND and JUDITH L. LUCAS, Boston, Mass. (Introduced by Benjamin Alexander).

The metabolism of cholesterol has been studied in two patients with angina pectoris before and after the induction of myxedema by I-131. C-14 labelled cholesterol was administered intravenously, and the decrease in specific activity of plasma cholesterol was followed for 149 to 300 days. The specific activity of the plasma free and total cholesterol, the red blood cell cholesterol and stool radioactivity in myxedema have been compared with the values in the same patient when euthyroid.

The induction of myxedema resulted in a rise in serum cholesterol from approximately 235 and 400 mgm. per cent to 400 and 650 mgm. per cent, respectively. In both patients, there was an initial period of about 13 days of relatively rapid decrease in plasma cholesterol specific activity. During this period, the disappearance curves of C-14 activity in the same patient when euthyroid and when myxedematous were quite similar. For the duration of the experiments, the rate of diminution of cholesterol specific activity in myxedema was much slower than in the euthyroid state. The half-time of disappearance from 30 to 104 days after infusion increased, in Case I, from approximately 65 days when euthyroid to 160 days following the induction of myxedema, and in Case II, it increased from 68 to 100 days; estimates of the size of the "exchangeable cholesterol pool" showed no change. The effect of the administration of desiccated thyroid and of changing cholesterol levels on the subsequent course of cholesterol radioactivity will be discussed.

The time for equilibration of labelled plasma free cholesterol with red blood cell cholesterol did not change in myxedema. On the other hand, the rate of appearance of C-14 labelled plasma cholesterol esters was much slower in myxedema than during the euthyroid state.

The data are regarded as consistent with decreased synthesis of cholesterol and cholesterol esters in myxedema.

ADH and Impaired Water Tolerance: Implications of Alcohol Diuresis in Certain Disease States. EZRA LAMDIN, CHARLES R. KLEEMAN, MILTON E. RUBINI, and FRANKLIN H. EPSTEIN, New Haven, Conn. (Introduced by John P. Peters).

Increased production, or diminished destruction, of posterior pituitary hormone (ADH) have been invoked to explain the impaired water metabolism characterizing

certain diseases. Experimental support for this hypothesis is, however, scanty. To assess the role of the posterior pituitary in such states, ethyl alcohol, a known transient inhibitor of ADH release, was imbibed by recumbent patients who failed to excrete normally a standard water load. Diagnoses included: cirrhosis, cor pulmonale, and congestive failure (with hyponatremia, edema, and/or ascites); obstruction of the superior vena cava; Addison's disease; and hypopituitarism. Alcohol was also given to water-loaded normal subjects in the head-up tilted posture—a state in which antidiuresis supervenes despite hypotonic expansion of body fluids.

Single doses of ethanol (33 to 55 grams), which provoke water diuresis in semirecumbent normals, did not affect the antidiuresis of the upright-tilted posture, and produced no increased urine flow in 10 of 11 recumbent patients.

Multiple doses (33 grams at hourly intervals) were used to prolong the inhibitory effect of ethanol on the neurohypophysis. After the second or third dose, the antidiuresis of upright-tilting was overcome, urine flow approaching diuretic values observed in the supine position before tilting. Of two cirrhotics with ascites given multiple doses, one responded equivocally, while the other, responding to the first dose with slightly increased urine flow, diuresed briskly after the second.

Conclusions: 1) Stimuli unrelated to tonicity of the extracellular fluids provoke the release of ADH from the pituitary; 2) Although other factors are undoubtedly operative, a diuretic response to alcohol implicates ADH as at least partly responsible for the impaired water excretion characterizing certain diseases; 3) Varying responses to the inhibiting action of alcohol suggest that the magnitude and intensity of this action of ADH probably differs from person to person and from disease to disease.

The Relation of Age to Certain Measures of the Performance of the Heart and the Circulation. MILTON LANDOWNE,* MARTIN BRANDFONBRENER, and NATHAN W. SHOCK, Baltimore, Md.

Estimates of resting circulatory performance have been made in a series of 67 men clinically free of cardiovascular disease, aged 19 to 86. Peripheral resistance (R) increased with age, as evidenced by a decline in cardiac output measured by dye dilution, unassociated with a decrease in mean brachial intra-arterial pressure. With respect to the heart, there was an agewise decline in estimated external left ventricular work (W) at rest, since the increase in mean systolic pressure was not comparable to the decrease in stroke volume, and the heart rate decreased. The increase in estimated systolic duration implies a further decrease in power (P), *i.e.*, rate of work performance. While the reduction in work may reflect lessened circulatory requirement, heart size did not diminish, and diastolic duration was increased. These indications of a decreased stroke work relative to diastolic volume specify a less favorable cardiac performance and therefore are evidences of a reduction in the reserve of the left ventricle as a muscular pump.

Estimated effective central elasticity (E) showed a striking age correlation ($r = .76$), as the relative rate of pressure fall in diastole increased with age, despite the increase in peripheral resistance. Residual individual variability in elasticity of ± 30 per cent limits the accuracy of predicting stroke volume from blood pressure curves by means of an age dependent factor for elasticity. Convenient approximations describing over-all results are R (mm. Hg min./Liter) $= 18e^{(.011 \pm .0014)a}$; W (gm. m./beat) $= 108e^{-(.005 \pm .0018)a}$; P (watts) $= 3.9e^{-(.009 \pm .0017)a}$; E (dynes/cm.^{5}) $= 900e^{(.016 \pm .0017)a}$ where a = age minus 50 yrs.

Effect of Folic Acid on Serum Vitamin B_{12} Concentrations in Pernicious Anemia. ARNOLD A. LEAR, Boston, Mass. (Introduced by Shu Chu Shen).

Therapy with folic acid alone may have a neuropathic effect in pernicious anemia, in which during relapse the serum vitamin B_{12} level is characteristically low. A study was therefore made of the effect of folic acid on serum vitamin B_{12} concentrations.

Twenty-seven patients with pernicious anemia who had been receiving 15 μg. of vitamin B_{12} intramuscularly every four weeks for two or more years were selected. During the first six months, 20 were given 30 μg. and 7 were given 20 μg. of vitamin B_{12} intramuscularly every two weeks. During the next six months, all received the same vitamin B_{12} dosage and in addition took 5 mg. of folic acid by mouth daily. Serum vitamin B_{12} levels were estimated microbiologically, using *Euglena gracilis*, initially and after 6 and 12 months.

Initially 14 of the 27 patients had serum vitamin B_{12} levels less than 100; average 202; range, 10 to 1050 $\mu\mu$g. per ml. After the first six months, only one patient had a level of less than 100; average, 451; range, 97 to 1360. After the second six months (supplemental folic acid), the average level was 279; range, 116 to 745. At this time, individual serum vitamin B_{12} levels in 18 of the 27 patients were 15 per cent or more lower than after the first six months. Results indicate that injection of 15 μg. of vitamin B_{12} every four weeks may be insufficient to saturate body vitamin B_{12} stores. Administration of folic acid may lower the serum vitamin B_{12} level possibly by accelerating the utilization of vitamin B_{12}. No neuropathic effects were observed.

The Volume of Distribution and Turnover of Endogenously Labelled Human Thyroid Hormone from Euthyroid and Hyperthyroid Donors. RICHARD P. LEVY, LUTHER W. KELLY, JR., GRAHAM W. COOPER, and WILLIAM MCK. JEFFERIES, Cleveland, O. (Introduced by Walter H. Pritchard).

The fate of endogenously labelled thyroid hormone obtained from two euthyroid patients has been compared with that obtained from two hyperthyroid patients, when administered to recipients in a euthyroid state. Recipients included subjects with normal thyroids which were

blocked by methimazole, preventing reutilization of I^{131}, and an athyroid patient maintained in a euthyroid state with desiccated thyroid. Serum PBI, total I^{131}, PBI^{131}, and urinary and fecal radioactivity were followed. From this data the volume of distribution and turnover rate of labelled hormone, the total exchangeable hormonal iodine, and the total daily hormonal iodine turnover were calculated.

It was found that approximately 24 to 30 hours were required for equilibration of the tracer hormone with the exchangeable hormonal pool, regardless of the donor. After this the rate of urinary plus fecal excretions equalled the rate of plasma fall in radioactivity, indicating equilibrium with an ultimate volume of distribution. When labelled hormone from a hyperthyroid donor and from a euthyroid donor was administered to the same recipient on different occasions, the daily hormonal iodine turnover rates were practically identical, although the volumes of distribution were different. When labelled hormone from the same hyperthyroid donor was administered to two recipients the volumes of distribution were similar, but the turnover rates were different. In one instance the volume of distribution attained by tracer hormone from a euthyroid donor was greater than that reached by tracer hormone from a hyperthyroid donor, in another it was smaller.

Hence the source of labelled hormone did not consistently influence its behavior in the recipients. It also appears that daily thyroid hormonal iodine turnover (utilization) may be the same in a single subject with repeat studies under similar conditions, whereas different euthyroid persons may exhibit rates of utilization which vary over a considerable range.

The Effects of Thyroid Hormone on the Distribution Kinetics and Degradation of Iodo-albumin. C. G. Lewallen, J. E. Rall*, M. Berman, and H. Hamel, Brookhaven National Laboratory and New York, N. Y.

To assess the role of thyroid hormone in albumin metabolism, seven patients with thyroid carcinoma and myxedema were studied with I^{131} iodo-albumin. Plasma specific activity and urinary excretion were followed up to 60 days. Iodide or antithyroid agents were administered to exclude label from thyroid tissue.

The plasma and urine curves were resolved by graphic analysis into three exponentials. With more frequent early collections, five exponentials were obtained from the urine curves. Significant disparity between retention and plasma slopes revealed an interval essentially non-feedback pool. External counts over liver and thigh in three patients were confirmatory.

The model found to be compatible with the data and used for analysis was a three-compartment doubly-open system consisting of plasma in parallel with one fast and one slow extravascular compartment providing for internal non-feedback loss and for external loss through a delay pool. A complete solution of this model was possible in one study.

Four patients were studied while myxedematous and after administration of thyroid hormone. There was in myxedema an average increase in total exchangeable albumin of 31 per cent, in extravascular albumin of 65 per cent, in fractional rate of transcapillary transport of 90 per cent, and in fractional rate of feedback to plasma of 24 per cent. In myxedema, the fractional rate of degradation was reduced by 47 per cent.

The metabolic status of two patients was changed during tracer studies by withdrawing or administering triiodothyronine. A rapid and marked effect in the rate of iodo-albumin degradation was clearly shown.

In a donor-recipient experiment performed to insure biologically homogeneous iodo-albumin, kinetic analysis favored the rapidly exchanging extravascular compartment as the major site of degradation.

The studies indicate that thyroid hormone exerts a profound effect on compartment size, degradation rate, and transcapillary transport of iodo-albumin in man.

Mechanisms Regulating Aldosterone Production in Man. Grant W. Liddle, Frederic C. Bartter,* Leroy E. Duncan, Jr., Joan K. Barber, and Catherine Delea, Bethesda, Md.

Although aldosterone has recently been shown to play a part in renal sodium conservation in edematous patients, the factors governing its production have not been defined. Studies designed to elucidate these factors have been carried out in human subjects, maintained under controlled metabolic conditions. Aldosterone production was estimated from the urinary excretion of methylenechloride soluble material having aldosterone-like activity as assayed in adrenalectomized dogs. Other indices of adrenal cortical function were followed, as were body weight, serum, urine, and, in some, fecal electrolytes, and endogenous creatinine clearance. Results were as follows: Sodium deprivation, with weight loss, consistently increased aldosterone excretion. Administration of potassium chloride resulted in increases of aldosterone output in those subjects in whom it induced sodium loss.

In sodium-depleted subjects, expansion of body fluids with water given with Pitressin® Tannate produced decreases in aldosterone and potassium excretion and increases in sodium excretion despite hyponatremia, suggesting that volume changes and not changes in concentration or in total body sodium mediate the effect of sodium on aldosterone secretion. Expansion of the vascular compartment with albumin without concomitant increase in total body water did not consistently decrease aldosterone excretion.

Reports that aldosterone output is increased in edematous patients have been confirmed. Infusions of physiologic saline in such subjects produced marked decreases in aldosterone output. ACTH caused increases in aldosterone output, which were significant, albeit small in proportion to concomitant increases in 17-hydroxycorticoid output. Abrupt withdrawal of ACTH resulted in transient falls in aldosterone excretion to values below pre-ACTH levels. Large doses of corti-

sone did not decrease aldosterone excretion significantly in sodium-depleted subjects.

The Effects of Triiodothyronine on the Biosynthesis of Plasma Lipids from Acetate-1-C^{14} in Myxedematous Subjects. S. R. Lipsky, P. K. Bondy,* E. B. Man, and J. S. McGuire, Jr., New Haven, Conn.

The incorporation of acetate-1-C^{14} into the free and ester cholesterol and the triglyceride and phospholipid fatty acids of the plasma was studied in three women with severe myxedema before and after a single dose of triiodothyronine. A significant fivefold decrease in the utilization of acetate for cholesterol biosynthesis occurred in these patients in the untreated state when compared with a series of six "normal" subjects. Although the total plasma cholesterol was elevated in all cases the diminution in labelling was found to be a reflection of reduced hepatic synthesis rather than the effect of simple dilution by an increased pool of circulating cholesterol. In one patient followed for 17 days, a decrease in peripheral utilization of the cholesterol was noted as evidenced by some delay in the rate of disappearance of the labeled sterol from the plasma.

The formation of the fatty acid fractions from acetate was only slightly retarded in two of the three subjects. In the third patient whose plasma was grossly lactescent and contained abnormally large quantities of neutral fat, synthesis was significantly reduced.

After an interval, 0.5 mg. of triiodothyronine was administered to these patients. Two days later, when the BMR and plasma lipids were rapidly returning toward normal, the isotope studies were repeated.

The rate of cholesterol synthesis returned to normal in one individual, and was still delayed in the two other patients. Normal rates of fatty acid synthesis were now noted in all patients.

It is concluded that in myxedematous subjects there is a decrease in hepatic synthesis of cholesterol as well as a defect in the peripheral utilization of this substance. With treatment, normal disposal rates prevail, cholesterol levels in plasma decrease, and synthesis returns toward normal.

Studies on Protein Synthesis in the Liver. John W. Littlefield, Elizabeth B. Keller, Jerome Gross, and Paul C. Zamecnik,* Boston, Mass.

It is known that the initial *in vivo* incorporation of intravenously injected radioactive amino acids into rat liver proteins takes place largely in the microsome fraction of the hepatic cell (the submicroscopic particles), and that ribonucleic acid is concentrated in this cell fraction as well. We have separated the microsome fraction into two components: sodium desoxycholate-soluble material containing 85 per cent of the protein of the original microsome fraction, and sodium desoxycholate-insoluble material containing 15 per cent of the original protein and essentially all the original ribonucleic acid. Purest samples of the latter contain equal amounts by weight of protein and ribonucleic acid, and may be termed "ribonucleoprotein particles." Under the electron microscope, unfixed preparations of such samples appear as essentially homogeneous dense granules averaging 240 A in diameter; in the ultracentrifuge, suspensions of such samples migrate as a sharp peak with sedimentation constant of 47 S.

We have studied also the *in vivo* incorporation of intravenously injected DL-leucine-C^{14} and L-valine-C^{14} into the two components of the microsome fraction and the soluble protein of the rat liver cell. Maximal incorporation into the ribonucleoprotein occurs within a few minutes after injection, while the bulk of microsomal protein and the soluble protein of the cell are labeled more slowly and progressively. One can estimate from such data that only a very small fraction of the total amino acids in the ribonucleoprotein are turning over rapidly. With the cell-free incorporation system (Zamecnik and Keller) we obtained evidence that the labeling of ribonucleoprotein is not merely a rapidly established equilibrium between free amino acids and those incorporated but is an irreversible step in protein synthesis.

Antidiuretic Content of Rat Hypothalami Under Different Experimental Conditions. C. W. Lloyd,* and P. O. Oldford, Syracuse, N. Y.

Antidiuretic activity of hypothalami and pituitaries of rats has been measured under different experimental conditions. Moderate dehydration (48 hours) without starvation produced an increase in the hypothalamic antidiuretic material. Intracarotid hypertonic saline solution was necessary to cause a decrease in hypothalamic antidiuretic substance. Maintenance on a liquid diet produced a decrease of antidiuretic activity. The hypothalami of hypophysectomized animals contained very little antidiuretic material shortly after hypophysectomy but there was essentially the same amount as in intact animals after a longer postoperative interval. Dehydrating for 48 hours produced a decrease and hydration caused an increase in antidiuretic activity of hypothalami of hypophysectomized animals.

The hypothalami of animals with diabetes insipidus, resulting from removal of the posterior lobe with maintenance of anterior lobe function, contained less antidiuretic substance than was found in totally hypophysectomized animals after the same postoperative interval. The administration of hydrocortisone caused a very large increase in the antidiuretic substance of the hypothalami of intact, hypophysectomized and adrenalectomized animals although more steroid was required in adrenalectomized rats. These data are compatible with the hypothesis that antidiuretic substance of the hypothalamus plays a part in the release of ACTH by the anterior lobe.

Atypical Pressor Responses to Standard Exercise. Gordon A. Logan and Robert A. Bruce,* Seattle, Wash.

Variations in blood pressure response in cardiac patients during exercise testing have been analyzed for different cardiovascular diseases. Blood pressure data

were derived from sphygmomanometric recordings of brachial artery pressure at minute intervals during the resting, exercise, and recovery phases of a standard test requiring patients to walk for 10 minutes at 1.7 mph and 10 per cent grade on a treadmill ergometer. Incomplete periods of exercise, presence of auricular fibrillation, variations in complications, treatment and age of patients are recognized as factors limiting interpretation and comparison of results.

In 16 normal subjects, systolic pressure increased from 20 to 45 mm. Hg (mean 25) by the third minute of exercise, remained unchanged during further exercise and gradually returned to normal during the recovery period. Diastolic pressure remained unchanged throughout.

Forty-five patients with predominant mitral stenosis showed only 5 mm. Hg mean systolic rise on exercise. During early recovery there was significant rebound. whereas diastolic pressure remained constant throughout. Following surgery, exercise systolic pressure approached normal and recovery rebound was nearly eliminated. These post-surgical changes were not related to surgical benefit. Analysis related to degree of exercise intolerance, cardiac deceleration during recovery, and presence of auricular fibrillation was done.

Eleven patients with aortic stenosis showed similar systolic hypotension during exercise, and slight rebound during recovery.

Sixteen hypertensive patients showed normal rise in systolic pressure, despite widened pulse pressure, during exercise. Evaluation immediately after drug-induced acute relative hypotension showed reduction in systolic, diastolic, and pulse pressures and increase in exercise tolerance.

Slight differences were found preoperatively in 13 patients with coarctation of the aorta.

Significant systolic and diastolic hypotension was apparent in 18 patients with patent ductus arteriosus with incomplete alleviation following surgery in 4 patients.

Mean diastolic pressure was constantly slightly reduced in 16 patients with interauricular septal defect.

Relationship of Aldosterone in Urine to Sodium Balance and to Some Other Endocrine Functions. JOHN A. LUETSCHER, JR.* and ROBERT H. CURTIS, San Francisco, Calif.

Increased sodium-retaining activity has been found in urine extracts from patients with nephrosis, heart failure, hepatic cirrhosis, and toxemia of pregnancy, when urine sodium excretion is low. Sodium-retaining activity is diminished during diuresis when urine sodium increases. Aldosterone has been isolated from one active urine extract from a child with nephrosis: in the other extracts, a material of similar chromatographic behavior is associated with the sodium-retaining activity.

Standardized methods of extraction of urine yield between 1.5 and 3.0 μg. of aldosterone per day from urine of normal subjects on unrestricted diets. Aldosterone was not found in Addison's disease or after bilateral adrenalectomy.

The output of aldosterone is not significantly affected by situations associated with changes in output of 17-ketosteroids or 17-hydroxy-corticoids, for example, in hypopituitarism, myxedema, adrenogenital syndrome, certain patients with Cushing's syndrome and normals given ACTH. Elimination of dietary sodium is followed by increased aldosterone output without appreciable change in 17-ketosteroids or 17-hydroxycorticoids.

An unexpectedly high output of aldosterone in relation to urine sodium has been observed in sodium-losing nephritis and in congenital adrenal hyperplasia. In all other cases studied, an inverse correlation exists between daily output of aldosterone and of sodium in urine, both in health and in disease. Thus the output of aldosterone appears to reflect a normal mechanism for sodium conservation, which under an abnormal stimulus causes accumulation of sodium in certain phases of disease. The stimulus to increased secretion of aldosterone is not clearly defined. It is possible that increased blood and urine levels might result from decreased destruction of aldosterone, which is rapidly inactivated by human and rat liver slices.

The Physiological Role of Adrenal Salt Hormone (Aldosterone) in Man. JOHN P. MACLEAN, MIN CHIU LI, MORTIMER B. LIPSETT, BRONSON RAY, and OLOF H. PEARSON,* New York, N. Y.

Adrenalectomized humans remain eucorticoid and in salt balance when receiving 50 to 75 mg. of cortisone acetate daily, together with a normal diet. Data will be presented to demonstrate that these patients cannot achieve sodium balance with a sodium intake of 20 mEq. a day, and that a salt depletion syndrome occurs. In contrast, 4 hypophysectomized patients with intact adrenals, maintained on 50 mg. of cortisone actetate daily, achieved sodium balance by the fifth day, with an intake of less than 10 mEq. daily. A salt depletion syndrome did not occur after two weeks on this regimen. The hypophysectomies were judged functionally complete by separate studies: 1) Development of acute adrenal insufficiency within 7 days after cortisone was withdrawn; 2) Clinical and laboratory evidence of myxedema; 3) Absence of pituitary gonadotrophin (FSH) in the urine.

The effect of aldosterone has been studied in an adrenalectomized human. Cortisone was withdrawn and acute adrenal insufficiency developed in 36 hours. In a subsequent cortisone withdrawal study, aldosterone, 900 μg., was given intramuscularly in divided doses over a 36-hour period. At this time an identical picture of acute adrenal insufficiency was manifest.

These observations indicate that the secretion of adrenal salt hormone responds to changes in the electrolyte milieu without the necessary intervention of the pituitary gland. Thus, the adrenal salt hormone in man appears to be necessary for the prevention of sodium depletion during sèvere dietary restriction or excessive extrarenal losses. Its role, therefore, is minor in comparison with hydrocortisone which is necessary for life maintenance.

The Response to Water Loading in Renal Failure. E. GORDON MARGOLIN and JOHN P. MERRILL,* Boston, Mass.

To determine the responsiveness of patients with chronic renal impairment to water loading, a series of cases on near-constant solute intakes was studied. In most instances the fluids were administered by mouth over periods of 2 to 5 days, but in several cases the additional fluids were given intravenously. The adequacy of physiological adjustment to water-loading was roughly correlated with the degree of disease as estimated by the levels of blood urea nitrogen.

In general, it can be stated that the response to the water loads was blunted; that is, urinary concentration dropped slowly if at all, while urinary output rose gradually during the challenge and returned to control levels only slowly after withdrawing the extra fluid load. The gain in solute excretion by the water-loading was minimal and seemingly decreasing as the challenge continued. The most effective time of day for unloading extra water by these patients was in the early morning hours. The patients tended to gain weight due to positive water balance and to develop signs of water intoxication consisting of bloating, generalized discomfort, nausea and vomiting and loss of desire for water. The handling of individual solutes, particularly sodium, chloride, and urea, closely paralleled the over-all dilution of the urine; potassium and phosphate, however, failed to follow this pattern.

Study of the dynamics involved would implicate hypotonic expansion of plasma volume with resultant increase in GFR in the increased total solute output. The ability to dilute the urine under this stress suggests tubular function of some degree.

This study points out the limitations of excess water administration in the management of patients with chronic uremia.

Effects of Fasting and Feeding on Pathways of Hepatic Glycogen Synthesis. PAUL A. MARKS and BERNARD L. HORECKER, New York City and Bethesda, Md. (Introduced by Alfred Gellhorn).

Studies with fasted animals have shown that $C^{14}O_2$ incorporated into liver glycogen is distributed predominantly and equally into carbon atoms 3 and 4. This finding was interpreted as indicating that glycogen synthesis occurred by a reversal of anaerobic glycolysis. Recent evidence has suggested that alternate pathways of glucose metabolism exist and that these may play a role in glycogen formation.

It has been known that fasting markedly affects overall hepatic carbohydrate metabolism. The present studies provide evidence that 1) the pattern of CO_2 incorporation into glycogen differs in fasted and fed rats; and 2) pathways other than the formation of glycogen *via* phosphopyruvate contribute to glycogen synthesis. Liver slices from rats fasted or fed a 58 per cent glucose diet were incubated with lactate and $C^{14}O_2$. Glycogen was isolated and degraded to determine the distribution of isotopic carbon. While carbon atoms 3 and 4 become equally labelled in liver slices from fasted animals, in those from fed animals position 3 had a lower specific activity than position 4. When glycerol was included in the incubation medium with fasted rat liver slices, the distribution of tracer was similar to that found with fed rats. The lower specific activity of position 3 suggests that glycogen formation in *fed* rats involves a condensation of dihydroxyacetone phosphate derived from endogenous precursors (unlabelled) and newly formed 3-phosphoglyceraldehyde prior to the complete equilibration of these trioses. Fed rats, in contrast to fasted rats, have a large source of dihydroxyacetone phosphate precursors, *i.e.*, glycogen and triglycerides of fatty acids.

In most experiments, small amounts of isotope were also incorporated into carbon atoms 1 and 2, and, to a lesser extent, 5 and 6. This asymmetric incorporation of isotope cannot be explained by a simple reversal of anaerobic glycolysis and suggests that under certain conditions, other pathways play a role in hepatic glycogen formation.

Effect of Growth Hormone and ACTH on Plasma Phospholipid Levels and Phospholipid Turnover in Man. E. A. MCCULLOCH, C. J. BARDAWILL, A. BRITTON, and K. J. R. WIGHTMAN, Toronto, Ont. (Introduced by J. A. Dauphinee).

A study of phospholipid turnover in the plasma of a series of patients has been carried out, using P 32 as a tracer. The curve of specific activity of phosphorus in the phospholipid fraction at various intervals after the administration of P 32 has been found to be remarkably constant in a given patient when studied repeatedly, although individual differences among patients may be quite marked. This individual constancy allows each patient to act as his own control, and gives significance to changes in response to treatment which may not be very large.

We have found that opposite alterations in the specific activity curve and the total plasma phospholipid are produced by ACTH and growth hormone. Preliminary observations in acromegaly appear to confirm the growth hormone effect. This consists of a fall in total plasma phospholipids, and a lowering of the specific activity curve. To date this is the only consistent metabolic effect of administered growth hormone that we have been able to detect in the human. A study carried out in an adrenalectomized patient during treatment with cortisone and on another occasion with desoxycorticosterone acetate gave results comparable to those reported when adrenalectomized dogs were similarly treated. Treatment of the intact patient with ACTH produced a rise in the total plasma phospholipid, but did not alter the shape of the specific activity curve. This is taken to indicate a net increase in phospholipid turnover.

These changes are probably mediated in the liver, and their exact significance is not known. The suggestion might be put forward that the change occurring after growth hormone represents an anabolic effect

(after Zilversmit). So far we have not detected any such change in other endocrinopathies.

Skin as an Electrolyte Reservoir in the Experimental Nephrotic Syndrome. JACK METCOFF,* IRENA ANTONOWICZ, SILVESTRE FRENK, JOHN CRAIG, JORGE MARTNER, and JOHN JAMES, Boston, Mass.

A nephrotic syndrome was produced in 24 immature male rats by injection of aminonucleoside (9-6 dimethylamino purine, 3-d amino ribose) for 12 days. Characteristic features and renal lesions were similar to those of the nephrotic syndrome in children. Body composition measured directly by tissue analysis and indirectly by balance study, was compared with that in 24 pair-fed controls. Both groups received NaCl loads orally. Isotope (Na_{24}, and Br_{82} or Cl_{36}) was administered to animals in each group 24 hours before sacrifice.

NaCl loading caused marked salt and water retention with negative potassium balance in nephrotic rats, but virtually unchanged balances in the controls. After sacrifice, nitrogen, electrolyte, and isotope analysis was performed on whole depilated skin, whole carcass, viscera and muscle. Analytical data were referred to non-collagenous dry fat-free solids. Carcass showed relatively small increases in total water, sodium and chloride content. The potassium content of edematous muscle was unchanged, but the intracellular potassium concentration (based on chloride distribution) was significantly reduced due to shift of water intracellularly.

Skin, containing 16 to 18 per cent of total body solids, showed the most striking changes, accounting for more than ½ of non-ascitic edema and ⅓ of tissue retained isotope. The very large amounts of potassium, sodium, and chloride found in these skins could not be satisfactorily assigned to either the extra or intracellular phases by conventional calculations. The data suggest that either sodium or potassium, in addition to chloride, is "bound" in skin. The magnitude of these quantities may define a specific obstacle in the interpretation of balance measurements.

Studies on Destruction of Red Cells by Canine Autoantibodies in Normal Dogs and in a Dog with Naturally Occurring Auto-immune Hemolytic Disease. GERALD MILLER, FRANK W. FURTH, SCOTT N. SWISHER,* and LAWRENCE E. YOUNG,* Rochester, N. Y.

Extensive studies have documented a naturally occurring form of auto-immune hemolytic disease in a dog. The canine disorder is an exact counterpart of the disease occurring in human beings, manifested by profound anemia, spherocytosis, autohemagglutination and agglutinability of the red cells by antiglobulin (Coombs) serum. Temporary remission following administration of ACTH was accompanied by qualitative change of the panagglutinin in the dog's plasma and an *increase* in titer of the panagglutinin.

Exchange transfusion permitted collection of plasma with high content of panagglutinin. Effects of transfusion of this plasma were studied in four experiments, involving three normal recipient dogs. *In vitro*, the panagglutinin strongly sensitized recipient animals' erythrocytes for the antiglobulin test. Following transfusion, the recipients' cells were *not* sensitized for the antiglobulin reaction, but coating of these erythrocytes could be demonstrated by loss of ability to react *in vitro* with samples of the plasma infused. These "blocked" cells showed sphering and increased osmotic fragility, and their life span was shortened as determined by Cr^{51} survival in each of two normal dogs.

Studies on the diseased animal and normal recipient dogs suggest that spherocytosis and hemolysis may be conditioned by factors other than the amount of antibody demonstrable on the cells or in the plasma, and that the "autoantibodies" of the canine disease can initiate hemolysis in normal animals. Although the actual mechanisms of hemolysis initiated by these antibodies still await elucidation, it seems clear that these mechanisms are operative in normal dogs transfused with plasma from the diseased dog.

It is probable that the beneficial action of ACTH in the dog (and perhaps in humans) was not mediated through suppression of antibody formation, but in part, at least, through an effect on the erythrocyte-antibody reaction.

An Analysis of the Rate and Volume of Forced Expiration as a Measure of the Mechanical Nature and Extent of Physiological Impairment Caused by Disorders of Ventilation. WILLIAM F. MILLER, ROBERT L. JOHNSON, JR., and NANCY WU, Dallas, Texas. (Introduced by Edward L. Pratt).

The 0.5 second expiratory capacity and total vital capacity determined during the course of a single maximal expiratory effort are accurate expressions of both the rate and volume of displaceable air and thereby constitute measures of obstructive and restrictive ventilatory disturbances. In order to determine the extent to which these measures estimate not merely displaceable lung volumes but alveolar ventilation as well, the $\frac{0.5 \text{ sec. EC} \times 100}{\text{TVC}}$ (EC ratio) and TVC were compared with arterial pCO_2, indexes of intrapulmonary gas distribution and lung volumes in 150 normal subjects and patients with respiratory disorders.

Ventilatory disturbances were designated as *obstructive* when the EC ratio was decreased below 60 per cent while the TVC exceeded 80 per cent of predicted; *restrictive* when the EC ratio exceeded 60 per cent and the TVC was reduced below 80 per cent; and *combined* when both were reduced. The extent of the impairment of these functions reflected the magnitude of the ventilatory disturbance.

The results of classification and quantitative evaluation of ventilatory disturbances in this manner correlated well with measured lung volumes. In the obstructive group both the total capacity TC, (135 ± 35 per cent) and the residual volume, RV (149 ± 32 per cent) were increased. In the restrictive group the reduced TC (74 ± 11 per

cent) paralleled the reduction in the TVC while the RV was normal (94 ± 29 per cent). Combined disorders revealed reduced TC (87 ± 14 per cent) but the RV (136 ± 50 per cent) varied in a manner predictable by the EC ratio, depending on the extent of airway obstruction present.

Similarly alterations of the EC ratio correlated excellently with measures of alveolar ventilation: arterial pCO_2 ($r - 0.83$), index of intrapulmonary mixing ($r - 0.86$) and the ratio $\frac{RV}{TC}$ ($r - 0.91$).

Thus, analysis of the 0.5 sec. component of the rapid expiratory volume provides a precise evaluation of both the nature and extent of mechanical ventilatory defects as well as the degree to which alveolar ventilation is impaired.

On the Importance of Available Base on Acetazoleamide Induced Renal Excretion of Carbon Dioxide. HERSCHEL V. MURDAUGH, Durham, N. C. (Introduced by J. D. Myers).

In respiratory acidosis due to CO_2 retention it would appear desirable to seek an extrapulmonary route for CO_2 excretion. Past attempts to produce adequate urinary loss of CO_2 using the carbonic anhydrase inhibitor, acetazoleamide, proved disappointing perhaps because of lack of a sustained effect (acetazoleamide fastness) and production of a metabolic acidosis. In considering this failure of acetazoleamide the following questions materialized: (1) What effect does available base have on acetazoleamide fastness? (2) As the renal excretion of CO_2 is increased by acetazoleamide, is the pulmonary output of CO_2 actually decreased? and (3) Is therapy of CO_2 retention by acetazoleamide and sodium loading practicable?

In evaluating the effect of available base on acetazoleamide fastness the following studies were performed. The effect of acetazoleamide on total urinary CO_2 excretion was determined in normal human subjects in the control state, and the subjects then were made acetazoleamide fast either by receiving the rice-fruit diet plus acetazoleamide for five days or by ingestion of ammonium chloride. In such acetazoleamide fast states, total urinary CO_2 excretion was diminished following acetazoleamide, but the effect of the drug was restored by intravenous sodium lactate (14 grams in physiological saline). The data, in mM total CO_2 per min., are as follows: For the rice-fruit diet group, control—0.439 ± 0.059, after diet—0.181 ± 0.025, after sodium lactate—0.265 ± 0.046, after sodium lactate and acetazoleamide—0.522 ± 0.114; for the ammonium chloride group, control—0.523 ± 0.088, after ammonium chloride—0.180 ± 0.039, after sodium lactate and acetazoleamide—0.501 ± 0.152.

The second question was approached by determining pulmonary and urinary outputs of CO_2 before and after acetazoleamide plus sodium lactate, with caloric intake constant in control and test periods. Urinary total CO_2 excretion increased by 1.16 ± 0.05 (S.D.) mM per min., and pulmonary output of CO_2 decreased by 1.34 ± 0.21 (S.D.) mM per min.

With such encouraging findings the effect of acetazoleamide with sodium load in patients with CO_2 retention is now being evaluated.

A New Type of Antibody and a "New Hypothesis" for Antibody-Antigen Interaction. VICTOR A. NAJJAR* and JEAN FISHER, Baltimore, Md.

Antisera to yeast alcohol dehydrogenase were produced in rabbits by multiple injections given subcutaneously. The antibody reacted readily with the enzyme to form a precipitate (antibody-enzyme complex) which still possessed considerable enzymatic activity. The antibody did not react with liver alcohol dehydrogenase. A study of the kinetics of the precipitin reaction at 0° C. showed a fast rate followed by a much slower one proceeding in a linear manner with time. This indicated the presence of two distinct types of antibodies.

When antibody was added to a constant amount of enzyme a point was reached where all the enzyme was precipitated by a definite amount of antiserum. Further additions of antiserum, however, resulted in further precipitation of antibody and further inhibition of the enzymatic activity of the complex. This occurred even when antiserum additions were made after the supernatant had already contained many times the amount of antibody necessary to precipitate the enzyme. These observations led to the hypothesis that the two types of antibodies present were antibodies to the enzyme and antibodies to the complex. Injection of the complex resulted in antiserum which reacted with the complex faster than it did with the enzyme as measured by rate of turbidity formation and rate of inhibition of enzymatic activity.

It was possible to absorb the antibodies to the complex by exhaustive addition of the antigen-antibody until no further reaction occurred. Following this the addition of the (enzyme) antigen resulted in immediate precipitation of antibody.

Requirement for Antibody and Complement in Phagocytosis of Starch In Vitro. ROBERT A. NELSON, JR.* and JACQUELIN LEBRUN, Institut Pasteur, Annexe de Garches, France.

Starch granules have been employed widely as an inert substrate for *in vitro* studies on phagocytosis. In contrast to the concept that starch is inert the present data demonstrate that two components of "normal" serum are essential for phagocytosis of the starch granule. The heat-stable component of serum conforms to the definition of antibody since 1) it is a serum globulin; 2) it is specifically and rapidly adsorbed to starch at 0° and is not eluted by repeated washings with saline; 3) it appears in increased quantity in animals injected with starch; and 4) once combined with starch, it induces specific immunological phenomena, *i.e.*, opsonization, agglutination and immune-adherence. The heat-labile component of serum appears to be complement. By papain

treatment of the starch-antibody-complement complex, it is proved that both serum components act on the starch granule and not on the phagocytes.

The constituent of the starch granule which is antigenic has not yet been defined.

A brief survey has indicated that antibody to starch is found in "normal" sera from several different species, including man.

Studies on a Second Outbreak of a Newly Recognized Infectious Exanthem. FRANKLIN A. NEVA, Pittsburgh, Pa. (Introduced by I. Arthur Mirsky).

An epidemic of an unusual exanthem which occurred in Massachusetts, in 1951, has been reported recently. Evidence was presented indicating the etiological relationship between this disease and a group of viruses isolated from these patients.

The initial observations have been corroborated and extended by studies of a second outbreak of a similar clinical entity, in Pittsburgh, during 1954. At least eighteen cases occurred, during two weeks, in June, among thirty families in a residential suburb. Six additional cases were detected, from July to September, in households in four different areas of the city. Multiple infections in six households were observed with adults as well as children affected.

Clinically, children showed fever for one or two days which was either followed by, or associated with, a pink maculo-papular skin eruption lasting two to three days and evident over the face, trunk, and extremities. Systemic signs and symptoms were usually minimal. By contrast, systemic manifestations were prominent in adults with chills and fever, severe headache, and muscle aches and pains. A skin rash which appeared after defervescence was recognized only in four of the eight adults studied.

Viruses have been isolated, with tissue culture techniques, from eleven of thirteen patients, representing both the suburban epidemic and sporadic cases. Most isolations have been from feces, but in one patient the agent was recovered from the blood. Based upon tissue culture host range, the agents resemble the group of viruses isolated from the Boston outbreak. Preliminary serologic tests indicate that Pittsburgh patients developed neutralizing antibodies to both local and Boston strains of virus.

These findings confirm the existence of an infectious exanthem differing from the common exanthemata. They support, furthermore, indications of an etiologic relationship between this disease and the agents isolated from specimens obtained during the acute phase of illness.

The Relation of Valve Function to the Genesis of the Sharp First Sound in Mitral Stenosis. HENRY T. NICHOLS, WILLIAM LIKOFF, HARRY GOLDBERG, and PHILIP LISAN, Philadelphia, Pa. (Introduced by Joseph DiPalma).

This study defines the genesis of the sharp first sound of mitral stenosis by correlating the mechanical function of the deformed valve with the laws that determine the duration and pitch of sound.

Events in the closure of normal and stenotic mitral valves were recorded in a group of patients undergoing cardiac surgery. Duration and pitch of the first sound were tabulated independently in these same patients prior to operation. The time relation of the first sound to the ventricular isometric period was determined by: (a) recording sounds simultaneously with pressure determinations during right heart catheterization, or (b) recording sounds from the surface of the heart when left ventricular pressures were taken at surgery.

Two events in the closure of the normal valve, the contacting force of the cusps, and intra-atrial displacement of the leaflets, were modified in mitral stenosis. The force was decreased consistently, and displacement was more abrupt or was eliminated entirely.

The first sound was sharp and delayed in relation to the isometric period when ballooning was abrupt. This type of valve acoustically functions as a two-dimensional stretched membrane, and it was concluded that the sharp, short sound ensued because of the decreased length and mass, and increased inertia and tension of the leaflets.

The first heart sound was normal in duration, pitch, and timing when extensive leaflet calcification prevented intra-atrial displacement. This type of valve functions acoustically as a diaphragm in which elasticity alone determines pitch.

Following mitral commissurotomy, the alteration in the sharp first sound is related to a buffered ballooning effect not to the size of the orifice. A sharp sound may appear when it was not present, if the valve is converted from a diaphragm to a two-dimensional stretched membrane with abrupt leaflet displacement.

The investigation suggested that the manner and degree of intra-atrial displacement is the mechanical event in the genesis of the sharp sound, and is an expression of changes which determine the vibratory qualities of the valve.

The Mechanism of Ammonia Excretion. JACK ORLOFF and ROBERT W. BERLINER,* Bethesda, Md.

Ammonia excretion is conditioned by factors involving intracellular production and net transport into urine. The urine pH is the major determinant of transport in the case of ammonia and certain exogenous weak bases. Thus it is possible to distinguish factors influencing ammonia production from those influencing transport by comparing the simultaneous excretion of ammonia and specific organic bases. Infusion of amino acid precursors of ammonia markedly enhanced ammonia excretion and increased urine pH; the decrease in titratable acidity was less than the rise in ammonia excretion. These data together with demonstration of a reproducible relationship between urine pH and rate of ammonia excretion when no substrate was administered, indicate that NH_3 diffuses into the urine and accumulates as non-diffusible NH_4^+. The weak bases can be considered to enter the urine by a similar process. The rate of excretion of ammonia

was found independent of urine flow when urine was acid but not when urine was neutral or alkaline. This is interpreted as indicating that ammonia enters the urine at a rate limited by ammonia production throughout the acid range. At higher pH's rate of production is non-limiting and rate of excretion is dependent on equilibration of the ammonia in tubule urine with that in tubule cells. Since the relationship between pH and rate of excretion extended into the most acid range, it is assumed that the more acid the urine the greater the length of tubule over which the urine is acid, and therefore the greater the segment of tubule which can contribute maximally to ammonia output. Since rate of excretion rather than concentration was a function of pH even in the alkaline range, these substances must enter urine at a site proximal to final water transport.

Studies on Headache: The Appearance of the Bulbar Conjunctiva Vessels in Patients with Vascular Headache of the Migraine Type. ADRIAN M. OSTFELD, New York, N. Y. (Introduced by Harold G. Wolff).

Observations of the bulbar conjunctiva in patients during headache and headache-free periods were made using a Poser slit lamp at a magnification of 47.5 × and camera attachment. Twenty seven patients were observed on one hundred and sixty occasions, and over two hundred photographs were made.

During the hours preceding headache an euvascular state was usual. Moderate arteriolar and venular constriction was also noted in some patients. During a migraine headache, dilatation of arterioles and venules, and increase in the number of patent capillaries were predictably noted on the headache side. Conjunctival edema was manifested by blurred appearance of tissue locally and indistinctness of the vascular structures. Slowed flow, sludging of blood, and venular hemorrhagic accumulation were frequently recorded. Vascular dilatation and conjunctival edema persisted as long as local scalp tenderness was present. Changes similar to those of migraine were seen unilaterally in two patients with atypical facial neuralgia. During subsidence of migraine headache as the result of intravenous infusion of nor-epinephrine, dilated arterioles constricted and showed increased segmental spasm. Also, edema disappeared. The caliber of venules was not immediately significantly altered.

Observations on patients who had had frequent and severe unilateral headache for many years exhibited tortuosity and moderate dilatation of venules which persisted even during headache-free periods. In muscle contraction headache, constriction of relevant arterioles and venules with blanching of capillary beds was observed. Arterioles showed striking segmental spasm and flow was slowed in vessels of all calibers.

Cranial artery pressure pulse waves were obtained using a glycerine pelotte, piezoelectric pulse wave attachment and direct writing electrocardiograph type recorder. High amplitude smooth contoured pulse waves from temporal and supraorbital arteries indicative of arteriolar dilatation accompanied conjunctival minute vessel dilatation during migraine headache. Low amplitude pulsations with increased number and size of reflected waves indicating vasoconstriction accompanied constriction of conjunctival minute vessels during muscle contraction headache.

The Effects of Isotonic and Hypertonic Salt Solutions on the Renal Excretion of Sodium. SOLOMON PAPPER, LAWRENCE SAXON, and HENRY W. COHEN, Boston, Mass. (Introduced by Maurice B. Strauss).

The natriuretic response to acute sodium loading administered as isotonic saline and hypertonic saline was studied in eight normal subjects. It was hoped that in this manner some information concerning the relative importance of acute changes in filtered sodium and acute changes in extracellular body fluid volume, as stimuli for sodium excretion might be obtained.

While taking 137 mEq. of sodium chloride daily, each subject was studied as follows. After 2 hours of control observation, 338 mEq. of sodium chloride was given intravenously over a 90-minute period. The salt was given as 0.9 per cent saline on one occasion and 5.0 per cent on another. In five of the subjects an additional experiment was performed infusing the same quantity of sodium as an isotonic "balanced" chloride-bicarbonate solution. Observations were continued for 2½ hours after the infusion was stopped.

There was no significant change in creatinine clearance associated with salt loading. Serum sodium concentration was increased more when 5 per cent salt was used than when either the 0.9 per cent saline or the isotonic-balanced solution was administered. The 0.9 per cent saline evoked a natriuretic response equal to or greater than that caused by the infusion of 5 per cent saline in six of the eight subjects. In the other two subjects the strong salt solution produced the greater increase in sodium excretion. The isotonic-balanced solution was as potent a natriuretic stimulus in two of the five subjects, a definitely weaker stimulus in two and a questionably weaker stimulus in one.

These data are consistent with the view that both increased filtered load of sodium and increased extracellular fluid volume are stimuli for sodium excretion. Furthermore, isotonic saline was at least as effective as hypertonic saline, in inducing renal sodium excretion.

Dynamics of Acute Failure of the Left Ventricle During Neurogenic Hypertension. J. L. PATTERSON, JR.* and H. G. LANGFORD, Richmond, Va.

The dynamics of the acutely failing left ventricle were investigated by the induction of acute myocardial necrosis and sudden hypertension. Injections of zinc hydroxide (F. H. Meyers) were made into the left ventricular myocardium, in nine dogs, and hypertension induced by electrical stimulation of the cerebral motor cortex for 60 to 180 seconds. Pressures were measured with strain gauges before and after dextran infusion, and cardiac output by dye dilution. Five normal animals given dextran and 40 animals previously studied served as controls.

Despite necrosis of 15 to 50 per cent of the left ventricle, mean aortic pressure rose with cortical stimulation by 64 mm. Hg, compared with 80 mm. Hg in the controls, primarily due to systemic vasoconstriction since cardiac output was unchanged. Calculated cardiac work increased 21 per cent in the experimental animals, 42 per cent in the controls. Pulmonary arterial and capillary (wedge) pressures showed small rises in the experimental and control animals during stimulation.

After infusion of 20 to 30 ml. dextran per kilo., stimulation produced increases in aortic pressures up to 240 and 210 mm. Hg in control and experimental dogs. Cardiac output increased 45 per cent in both groups over predextran values. Dextran infusion resulted in slight wedge pressure rise in controls, with little further change during stimulation. In the experimental dogs, infusion alone produced 11 mm. Hg rise in capillary pressure. An additional 13 mm. increase on stimulation resulted in several pressures over 40 mm. Hg. One experimental animal had no increase in pulmonary capillary pressure but a 14 mm. Hg rise in left atrial pressure.

These observations show that a severely injured left ventricle can support acute hypertension and large increases in cardiac work for brief periods but at the price of high filling pressure. They further indicate that increase in blood volume plays a critical role in the phenomena of acute left ventricular failure.

Some Problems in Interpreting the Significance of Total Peripheral Resistance. LYSLE H. PETERSON, Philadelphia, Pa. (Introduced by R. D. Dripps).

The dynamics of hemodynamics is incompletely understood. One important aspect of the complex subject involves the interpretation of so-called total peripheral resistance. Its magnitude is commonly assumed to be given by the ratio: mean intra-aortic pressure minus mean "central" venous pressure/mean cardiac output. Even if that simple assumption were true, *i.e.*, no energy added by the skeletal and respiratory pumps, the *distribution* of the resistance is not indicated. The localization of the resistance is, however, important to an understanding of the physiology, pharmacology, and diseases of the cardiovascular system. One approach involves the measurement of pressure drop. For this to have meaning it would be necessary to measure simultaneously the blood flow as well as the pressure drop along all vessels in the cardiovascular system. Even if such a task were possible the summation of values would be further complicated by the fact that the resistances are in series, parallel, and series-parallel. We have used another approach, favored by physicists and engineers, to study complex circuit parameters including resistance. This method involves the analysis of the pressure pulse which results when an artificial stroke volume, of known parameters, is introduced into the aorta. By so doing we have been able to differentiate the resistance which is distributed along the arterial system from the total peripheral resistance. This is the pre-arterial or, more strictly the pre-elastic resistance. This resistance offered by the arterial system itself is least when blood pressure is "normal" but increases rapidly with hyper- or hypo-tension. We have concluded that the magnitude of these changes are such that prearteriolar resistance may have an important role in normal and abnormal cardiovascular behavior.

The Miscible Pool and Turnover Rate of Hydrocortisone in Man. RALPH E. PETERSON and JAMES B. WYNGAARDEN, Bethesda, Md. (Introduced by Harry Eagle).

Data on total daily adrenal hydrocortisone production have not been available. Attempts to estimate its production by measurement of urinary steroid metabolites of hydrocortisone have been unsuccessful because of inadequacies of hydrolytic and colorimetric methods. Applying conventional tracer techniques, we have administered hydrocortisone-4-C^{14} intravenously in tracer amounts in 10 per cent ethanol (200 to 500 micrograms; 1 to 2.5 microcuries). Plasma samples were collected over 4-hour period and extracted with dichloromethane. The dichloromethane was evaporated dry, the residue applied to paper and chromatographed in a Bush-type system. Hydrocortisone was eluted, and its specific activity determined by assaying aliquots for radioactivity and hydrocortisone by sulfuric acid-ethanol fluorescence. From linear semilogarithmic plots of specific activity *vs.* time, the metabolic pool of hydrocortisone and rate of turnover was calculated. In eight studies in five normal subjects the pool of hydrocortisone ranged from 1.3 to 2.4 mg. with turnover rates of 0.30 to 0.67 pools per hour (0.66 to 1.10 mg. per hour). Projected to daily endogenous hydrocortisone production, figures of 16 to 26 mg. per day were obtained, with mean of 21.3 mg. Hydrocortisone spaces, calculated from pool size and plasma concentration, were 15 to 20 liters. Two patients with cirrhosis of the liver had turnover rates of 4.7 and 7.2 mg. per day, with increased pool in one with ascites. Two patients with hypothyroidism had turnover rates of 3.5 and 13.8 mg. per day. A normal subject under influence of continuous maximal ACTH stimulation had pool of 9.07 mg., with turnover of 181 mg. per day. In normal subject given 60 mg. metacortandracin over 36-hour period prior to study, hydrocortisone production 8 to 12 hours after the last dose was 1.2 mg. per day. This method offers promise for more precise measurement of adrenal function.

The Effect of Carbon Dioxide on the Concentration of Calcium in Ultrafiltrate of Serum Obtained by Centrifugation. ANANDA PRASAD and EDMUND B. FLINK, Minneapolis, Minn. (Introduced by F. W. Hoffbauer).

An ultrafiltrate of serum was obtained by centrifugation of serum in Visking® casing suspended in gauze bags in large centrifuge tubes. Centrifuging 10 ml. serum at 2500 r.p.m. for 75 minutes produced 2.5 to 3.0 ml. ultrafiltrate. The blood was collected and serum separated anaerobically using mineral oil seal. The Visking® casing and centrifuge tube for its suspension were flushed

with 5 per cent carbon dioxide and 95 per cent oxygen (I). In other experiments blood was drawn without anaerobic precautions (II) and in a third set of experiments the serum was saturated with carbon dioxide by bubbling the same mixture through the serum or by using higher concentration of carbon dioxide for flushing the centrifuge tube (III). Calcium was determined by the Kramer and Tisdall method both on serum and on ultrafiltrate. pH was determined on the serum residue in the casing at the end of centrifugation in (I) and (III).

When the diffusible calcium in normals is expressed as per cent of total calcium the mean and range of values in the three procedures are as follows: 53.3 per cent (47 to 56) in (I); 52 per cent (42.7 to 58.4) in (II); 64.2 per cent (57 to 72) in (III).

There is a significant increase in diffusible calcium when the serum is exposed to an excess of carbon dioxide (III) although the pH is kept in the same range as I. This suggests that the excessive carbonic acid produces an artefact and that the normal diffusible calcium expressed as per cent of total calcium actually is 53 per cent ± 5.

Relationship of Corticotropin, Intermedin, and Growth Hormone to Metabolic Activities of Pituitary Extracts. M. S. RABEN * and E. B. ASTWOOD,* Boston, Mass.

Purification of pituitary extract by adsorption on oxycellulose yielded a product potent in corticotropin, intermedin, and extra-adrenal metabolic effects concerned with fat and carbohydrate metabolism. The product did not contain growth hormone, which was concentrated from the fraction not adsorbed by oxycellulose. Fractionation of the oxycel-adsorbed product on columns of carboxylic resin or oxycellulose, or by countercurrent distribution or, less satisfactorily, by precipitation methods, separated a fraction, constituting about 10 per cent of total, of greater intermedin potency and greatly diminished corticotropic and metabolic activity, and a corticotropic-metabolic fraction with much diminished intermedin activity. The latter fraction did not yield to attempts to separate the corticotropic from the metabolic activity and from the residual intermedin activity and no differences in susceptibility of the corticotropic and metabolic activities to inactivation were found. Treatment with 0.1N NaOH at 100°C. increased intermedin potency 10 to 30 times (tested in *Rana pipiens*) and greatly increased duration of action. Treatment for 1 minute potentiated intermedin without appreciable loss of metabolic activity; after 15 minutes, corticotropic and metabolic activities were largely lost while intermedin activity was still enhanced. Metabolic and intermedin activities were also found present in alpha-corticotropin, corticotropin-beta, and corticotropin-B, fractions isolated from the oxycel product in three different laboratories. Both oxycel material and purified growth hormone preparations caused in mice increased liver fat, ketosis, lowering of blood sugar, and protection against insulin hypoglycemia, but the oxycel fraction was many times the more potent. The oxycel fraction was not found to be diabetogenic in the dog, while growth hormone preparations were diabetogenic but the ratio of growth to diabetogenic activity varied widely. The overlapping activities which were found may result from inadequate separation of contaminants but the possibility is also considered that the several metabolic activities of corticotropin and growth hormone are side effects of obscure physiological significance.

The Effects of Hydrocortisone on Water Diuresis and Renal Function in Man. LAWRENCE G. RAISZ, WILLIAM F. MCNEELY, LAWRENCE SAXON, and JACK D. ROSENBAUM,* Boston, Mass.

An enhanced diuretic response to water in subjects receiving cortisone has been previously described. In order to investigate the mechanism of this change the acute effects of a 5 to 6-hour intravenous infusion of 200 mg. of hydrocortisone were observed in three subjects during maximal water diuresis. In two of these, serial water diureses were studied before, during, and after the oral administration of 160 to 280 mg. of hydrocortisone daily for 10 to 14 days. The influence of oral cortisone, 300 mg. daily, was similarly studied in another subject. Each study included determination of electrolyte excretion, clearances of inulin (GFR), paraaminohippurate (ERPF), total osmols (C_{osm}) and "free" water (C_{H_2O}). All subjects were healthy men, maintained on low sodium diets.

During oral hydrocortisone administration maximal water diuresis increased progressively. By the second week C_{H_2O} had risen from 9 and 16 ml. per min. to 17 and 23 ml. per min. in the two subjects, and the per cent of filtered water excreted from 10 and 14 to 16 and 19 per cent, respectively. GFR and ERPF increased irregularly to maximal values 10 to 20 per cent above control levels. Sodium excretion, C_{osm}, and body weight all increased slightly. After hydrocortisone was discontinued C_{H_2O} returned to pretreatment values in 3 to 5 days. Similar changes were observed with oral cortisone.

Intravenous hydrocortisone produced smaller increases (2 to 4 ml. per min.) in C_{H_2O} with a larger rise in GFR and decreased sodium excretion.

The observed increase in C_{H_2O} without fall in C_{osm} implies increased delivery of water and solute to the distal tubule where the extra solute, but not the water was largely reabsorbed. Such an increased delivery could be a consequence of increased GFR alone; however, the lack of correlation between changes in GFR and C_{H_2O} indicates an additional direct effect of hydrocortisone to diminish proximal reabsorption.

The Effect of Clotting on the Spontaneous Activation of Plasmin. OSCAR D. RATNOFF,* Cleveland, O.

The biological significance of the proteolytic activity which is potentially available in human plasma is undetermined. Plasmin, the proteolytic enzyme or enzymes effective at neutrality, is capable of digesting fibrinogen, fibrin and other plasma components. However, in freshly shed blood, plasmin appears to be in an inactive form,

plasminogen. Under certain conditions *in vitro*, plasmin may become active "spontaneously," or upon addition of such agents as chloroform, tissue particles or streptokinase, a principle elaborated by hemolytic streptococci.

Earlier experiments demonstrated that the activation of plasminogen by streptokinase was greatly potentiated by blood coagulation. Evidence has now been obtained that the *spontaneous* activation of plasmin is also potentiated by the clotting process.

A globulin fraction of native plasma, prepared in silicone tubes without the addition of anticoagulant, remained fluid in silicone-coated tubes but clotted readily when mixed with crushed glass. This fraction contained fibrinogen, prothrombin, proaccelerin, proconvertin, plasminogen and many other substances. After 24 hours, the unclotted or clotted globulins were mixed with casein, and the amount of casein digested was determined in silicone tubes. Much more casein was digested by the clotted globulin than by the unclotted globulin. In control experiments, globulin prepared from serum instead of plasma developed no more proteolytic activity in glass than in silicone tubes, and no more than the same subject's unclotted native globulin in silicone tubes.

Thus it appeared that the clotting process activated plasmin from its precursor. This correlated with the rapid lysis of fibrin which occurred in the globulin which had been clotted in the presence of glass, compared with the stability of fibrinogen in the unclotted globulin kept in silicone. These experiments suggest that the proteolytic activity of plasma may be activated when clotting occurs, permitting the destruction of fibrin. The possible importance of this phenomenon *in vivo* is apparent.

Modification of the Gastric Secretory Response to Histamine During Varying Behavioral States: Observations on an Infant with a Gastric Fistula. FRANZ REICHSMAN, GEORGE L. ENGEL,* and HARRY L. SEGAL, Rochester, N. Y.

It is generally believed that histamine acts directly on the peripheral secretory mechanism of the stomach. There is some evidence that the magnitude of response to a standard dose of histamine is related to the peripheral reactivity of this secretory mechanism, as influenced by stimuli arriving *via* normal physiological channels.

Observation of a 1½ year infant with a congenital atresia of the esophagus and a gastric fistula provided an opportunity to examine the relation between behavioral states and gastric secretion, including the responses to histamine. Over a period of five months, behavior and gastric secretion were studied during 59 observation periods, a total of 161 hours, yielding more than 600 specimens of gastric juice. Histamine phosphate, 0.1 mg. per 10 kg. of body weight was administered subcutaneously nine times, yielding 55 specimens of gastric juice.

In general it was found that the mean rate of secretion of total HCl (mEq. per min.) when the child related actively to the experimenter in a friendly or aggressive way was more than twice as much as when she was withdrawn, depressed, or asleep ($P < 0.01$). Histamine actively stimulated HCl secretion during the outgoing states, but was relatively ineffective during the depressed, withdrawn state or sleep. Thus, in three experiments when relating actively to the experimenter the total secretions of HCl were 2.21, 2.35, and 2.39 mEq. in an hour; in three experiments when withdrawn, 0.16, 0.49, and 0.54 mEq. in an hour. When the 55 specimens of gastric juice were classified according to the behavior manifest during the period of collection of each specimen, the same relationship was established. Thus, specimens obtained during periods of withdrawal, depression, and sleep revealed significantly lower rates of total HCl secretion (mEq. per min.) than those during an active relationship with the experimenter ($P < 0.01$).

The Nephropathy of Potassium-Depletion: A Clinico-Pathologic Entity. ARNOLD S. RELMAN* and WILLIAM B. SCHWARTZ,* Boston, Mass.

A study of five adults with severe chronic potassium-depletion has defined the clinical and pathological features of a previously unrecognized renal disorder apparently caused by potassium deficiency.

Potassium-depletion in each instance had resulted from chronic diarrhea of various etiologies. Urinary excretion of potassium, measured in four patients, was below 10 mEq. per day, indicating efficient renal conservation. Severe hypokalemia was the rule, but no patient was dehydrated or sodium-depleted, and none had significant acid-base disturbances. One patient noted marked polyuria and polydipsia, and two had intermittent ankle edema. Routine urinalyses revealed minimal inconstant albuminuria and cylindruria in three.

BUN and serum creatinine were normal or only slightly elevated, but PSP excretion was low, and all patients exhibited Pitressin®-resistant hyposthenuria. Clearances of inulin, endogenous creatinine and PAH were significantly reduced. In the one patient from whom renal venous blood was obtained, the PAH extraction ratio and Tm PAH were low.

Full replacement of potassium deficits in three patients resulted in gradual restoration of normal renal function in two, and nearly complete recovery in the third. Potassium replacement was incomplete in the remaining two patients; renal function was unimproved after a year in one, while in the other concentrating ability improved but the clearances of inulin, creatinine, and PAH did not.

Renal biopsies were obtained in the latter two patients. In each case there were striking hydropic and degenerative changes, confined chiefly to the convoluted tubules, without significant glomerular or vascular disease. These lesions resemble the vacuolar tubular changes described by earlier workers in patients dying of various diarrheal diseases, and they are not unlike the renal changes produced by experimental potassium-depletion in rats. Despite absence of significant functional improvement following partial repletion, repeat biopsies showed distinct evidence of healing. This observation, taken together with the functional recovery in the other patients, suggests that the disease is probably reversible.

Effects of Hydrocortisone on Carbohydrate Metabolism of a Patient with Renal Glucosuria (DeToni-Fanconi Syndrome). ALBERT E. RENOLD, E. RUDOLF FROESCH, JOHN B. REARDAN, and JOHN T. FINKENSTAEDT, Boston, Mass. (Introduced by George W. Thorn).

The measurement of hormonal effects on carbohydrate metabolism in man is hampered by the efficient mechanisms which maintain constant blood glucose levels and nearly complete glucose reabsorption from the glomerular filtrate. Incomplete tubular reabsorption of glucose leads to a persistent drain on blood glucose which is proportional to the filtered glucose load. With incomplete tubular reabsorption, urinary glucose excretion in the fasting state has been assumed to reflect gluconeogenetic activity. The effects of hydrocortisone on glucose excretion have been studied in a patient with severe congenital renal glucosuria, regularly excreting 30 to 40 per cent of the filtered glucose load at blood glucose levels below 100 mg. per cent. Two requirements in methodology had to be met: (1) Urinary glucose determinations were made with a specific enzymatic method (using glucose oxidase) to eliminate interfering non-glucose reducing substances; (2) Glucose was enzymatically removed to facilitate inulin measurements in urine specimens containing large amounts of glucose. Hydrocortisone was administered intravenously to the fasting patient (25 mg. per hour for 8 hours). Inulin and creatinine clearances were determined throughout. Urinary glucose excretion rose from an average of 11 mg. per minute to a maximum of 37 mg. per minute. The greater portion of this increase could be related to the increased filtered glucose load and is assumed to represent increased hepatic gluconeogenesis in view of the progressive increase in blood glucose levels. In addition, however, *a definite decrease in tubular glucose reabsorption was noted* (from 18 mg. per minute to 7 mg. per minute). These changes will be related to nitrogen, phosphate, and potassium excretion. Similar studies in normal subjects will be reported and animal studies attempting to elucidate the mechanism of decreased tubular glucose reabsorption will be discussed.

Renal Excretion of Carbon Dioxide in Normal Subjects and in Subjects with Carbon Dioxide Retention Following Blockage of Bicarbonate Reabsorption in the Renal Tubule. ALBERT L. RUBIN and WARREN S. BRAVEMAN, New York, N. Y. (Introduced by Thomas P. Almy).

It is known that patients with carbon dioxide retention and respiratory depression may improve clinically after the administration of Diamox®, a carbonic anhydrase inhibitor. In association with this improvement, arterial pCO_2 falls. The pCO_2 of the urine has been observed to rise as renal bicarbonate excretion increases. If the rise in urinary pCO_2 were of sufficient magnitude, it might account for the fall in arterial pCO_2 seen in these patients with chronic carbon dioxide retention after Diamox® is given.

Urinary excretion of sodium, potassium, bicarbonate, and chloride ions, and of carbon dioxide was measured in six normal subjects, and in five subjects with chronic carbon dioxide retention, before and after the administration of Diamox®.

A strikingly similar pattern of response was obtained in the six normal subjects. After Diamox® administration, all exhibited a rise in urinary pCO_2 which paralleled the increased excretion of bicarbonate ion. The six patients with chronic carbon dioxide retention, all of whom improved clinically and showed a significant fall in blood pCO_2 after Diamox® was given, exhibited increases of urinary pCO_2 of the *same* order of magnitude as the normal subjects. This is not of sufficient magnitude to account for the fall in arterial pCO_2 that is observed. Therefore we believe that renal excretion of carbon dioxide is not a significant factor in lowering the arterial pCO_2 in patients with carbon dioxide retention given Diamox®. Some other compensating mechanism must be postulated.

Glutamic-Oxalacetic Transaminase Levels in Experimental Tissue Damage. L. A. RUDOLPH, R. DUTTON, and J. A. SCHAEFER, Syracuse, N. Y. (Introduced by R. H. Lyons).

This study involved investigation of serum glutamic-oxalacetic transaminase levels, using the method described by Karmen, in dogs subjected to infarction and ischemia of various organs. Venous blood was drawn daily before and after each surgical procedure. Eight animals were subjected to myocardial infarction, while branches of the pulmonary artery were ligated in six. Ligation of the renal, splenic, and mesenteric vessels was done in groups of three dogs. Elevations of serum transaminase activity in peripheral venous blood in these studies were characterized by peak levels within twenty-four hours which fell promptly to normal, with the exception of ligation of the lobar vessels where a secondary rise was noted.

Those experiments which produce infarctions involving small areas of myocardium did not always result in increased activity in the peripheral blood above the normal range. There was a similarity between the pattern of response in these experimental infarcts and the clinical cases of myocardial infarction. Generally, there was a rough correlation between the degree of tissue involvement and level of enzyme activity.

Nine animals were subjected to temporary occlusion of splenic, renal, pulmonary, mesenteric, and coronary vessels for short periods of time. Ischemia of this short duration produced no elevation of levels in the peripheral blood.

This study suggests that increases in serum enzyme activity are a manifestation of infarction of the tissues studied, which are not a characteristic of any particular organ.

Evidence for Circus Movement in Atrial Flutter in Man. DAVID A. RYTAND,* DAVID L. BRUNS, and SILVIO J. ONESTI, JR., San Francisco, Calif.

If the mechanism of human atrial flutter involves rapid stimulation from an ectopic focus uninfluenced by return-

ing excitation, the rate probably should be relatively independent of atrial size. But if the mechanism includes a continuing wave of excitation (circus movement), the rate should be reduced by (a) slower conduction velocity or (b) lengthened pathway as might accompany atrial dilatation.

The atrial flutter rate is relatively slow in patients with right (but not left) atrial dilatation. In 7 patients in this clinic and 9 more obtained from the literature and by personal communications, the mean rate with right atrial dilatation was 210 per minute (cycle, 0.28 sec.). Without dilatation, 93 other patients with flutter had a mean atrial rate of 284 (cycle, 0.21 sec.). The difference of 0.07 sec. in cycle duration is statistically significant. Patients receiving quinidine were excluded, as were those without electrocardiographic undulations typical of flutter as distinct from paroxysmal atrial tachycardia.

Evidence was obtained which favored lengthened pathway of a hypothetical circus wave rather than depressed conduction velocity as an explanation for this clinical finding. Observations with esophageal electrodes in 2 patients with flutter and dilated atria yielded velocities of approximately 125 and 167 cm. per sec. as the wave travelled upward, even more rapid than values about 84 cm. per sec. found similarly in others with flutter but without atrial dilatation. Such acceleration of velocity, expected with longer pathways, is contradictory to the depression predictable if atrial anoxia or high intra-atrial pressure were the cause of the observed slow rates.

Finally, exploration of the anterior chest walls of these 2 patients strongly suggests a downward descent of excitation, as if the wave were returning.

These observations represent evidence in support of the circus movement hypothesis as a mechanism in human atrial flutter.

A Physiological Explanation for Results of Water Loading Tests. F. SARGENT, II, R. E. JOHNSON,* A. A. PANDAZI, I. J. LICHTON, and T. W. NIELSEN, Urbana, Ill.

To investigate whether or not water loading tests can be used to estimate dehydration, simultaneous measurements were made of deuterium oxide space by a dilution technique after oral dosage, serum and urine osmotic pressures by freezing point depression, and urinary excretion of a test dose of water (20 ml. per kg. body weight; excretion measured for four hours; correction for basal excretion; subjects reclining, not smoking). For two weeks during a six-week study, 99 healthy young men in groups of four or more subsisted on controlled regimens of unlimited or limited water intake (910 ml. per day); low osmotic load (pure carbohydrate); high osmotic load (high protein—low salt, or moderate protein—high salt); or intermediate osmotic load (medium protein or medium salt). Measurements were made initially and again after 12 days. The water test dose was not retained in normally hydrated individuals, regardless of osmotic load; or in dehydrated, osmotically depleted subjects. It was retained in dehydrated subjects whose osmotic excretion was moderate to high. The hypothesis is defended that, in healthy subjects, the body excretes a test dose of water when the normal total body osmotic concentration is in danger of depression, but retains water when the osmotic concentration will not be depressed below normal by the test dose. The clinical implication of this hypothesis is that the response of a patient to the water loading test of Robinson, Power, and Kepler may be determined primarily by state of hydration and osmotic balance; and not specifically by endocrine or renal function.

The Survival of Normal Ceruloplasmin in Patients with Hepatolenticular Degeneration (Wilson's Disease). I. HERBERT SCHEINBERG,* DONALD T. DUBIN, and RUTH S. HARRIS, New York, N. Y.

Wilson's disease is characterized by a deficiency of a specific plasma protein, ceruloplasmin. This deficiency could be caused by an increased rate of destruction, or loss of the protein, on the one hand, or by a decreased rate of synthesis of the protein, on the other, in comparison with normal subjects. The present work was undertaken in an attempt to distinguish between these mechanisms.

Ceruloplasmin was administered by means of incomplete exchange transfusions to three subjects. Two of these were girls, aged 13 and 21, with Wilson's disease who possessed less than four per cent of the normal level of ceruloplasmin. The third subject, a healthy one-year-old brother of the younger girl, possessed less than two per cent of the normal level of this plasma protein.

The concentrations of ceruloplasmin which were attained in these subjects were determined at intervals following the transfusions. The half life time of the administered ceruloplasmin, calculated from these measurements, was 3.4, 4.4, and 4.7 days, respectively, in the one, 13 and 21 year-old subjects.

If the rate of synthesis (grams per day) of ceruloplasmin in any of these subjects were assumed to be the same as that in normal subjects, the latter would be required to have ceruloplasmin whose half life time was at least 110, 118, or 170 days in order to maintain normal plasma levels of the protein. Half life times of such magnitude are sufficiently greater than those known for other plasma proteins to cast serious doubt on their being correct. Therefore, there is probably a diminished rate of synthesis of ceruloplasmin in the subjects studied in comparison to the normal.

Effect of Salt Restriction on Sodium Excretion in Sweat. IRVING L. SCHWARTZ, NIELS A. THORN, and ALVAN R. FEINSTEIN, New York, N. Y. (Introduced by Vincent P. Dole).

The sodium concentration of human sweat is known to be reduced by administration of desoxycorticosterone and also by physiologic and pathologic hyperactivity of the adrenal cortex.

Previously we have presented evidence that sodium

excretion in sweat involves two processes, one, delivery of sodium into a precursor solution, the other, reabsorption from this precursor. We believe that both of these processes require cellular work because studies with weak electrolytes show that only the un-ionized molecular species can enter the sweat by simple diffusion. The question is, therefore, whether this steroid-induced reduction in sweat sodium concentration is due to diminished sodium input or enhanced reabsorption. The former implies reduced cellular work, the latter, increased.

The rate of sodium input into the precursor, I_{Na}, and the rate of reabsorption, R_{Na}, increase with rising sweat flow. Therefore, both I_{Na} and R_{Na} have been measured at a standard sweating rate of 10 grams M^2 minute. At this flow I_{Na} exceeds R_{Na} in all tubules; thus R_{Na} represents maximal reabsorptive capacity.

Four patients were studied during periods of high (175 mEq. per day) and low (5 mEq. per day) dietary sodium intakes. During the high sodium period, I_{Na} averaged 753 ($\pm$ 96.3) μEq. per M^2 minute and R_{Na} averaged 238 ($\pm$ 54.9) μEq. per M^2 minute. Restriction of sodium decreased I_{Na} to 343 ($\pm$ 48.2) μEq. per M^2 minute and also decreased R_{Na} to 78.5 ($\pm$ 19.0) μEq. per M^2 minute.

These findings, in conjunction with similar observations after administering desoxycorticosterone, suggest that the salt-active steroids decrease sodium reabsorption. The diminished input is of sufficient magnitude to lower the sodium concentration of the final product despite the decreased reabsorption. Thus, the steroids decrease the cellular energy expended for both the delivery and the reabsorptive processes.

The Precordial Electrocardiogram: Is it Predominantly Derived from the Heart Muscle Immediately Beneath the Electrode? GEORGE E. SEIDEN and ROBERT A. KEISMAN, Philadelphia, Pa. (Introduced by Thomas E. Machella).

It has been widely believed that a QRS complex recorded in a chest lead is predominantly derived from the electrical activity of the subadjacent portion of heart muscle. This assumption has been tested experimentally in a study of 10 normal subjects and 20 patients with heart disease and obvious electrocardiographic abnormalities, each with a QRS configuration or deformity peculiar to a localized area of the precordium. In each individual a mirror of the chest lead pattern was found at a site remote from the precordium. The precordial signal and its mirror were electrically "bucked" against each other with an appropriate cancellation technique. The quality of the cancellation is expressed by the residual voltage as a fraction of a weighted average of the two cancelling signal voltages. In no instance was the residual greater than 15 per cent. Stated otherwise the great preponderance of the precordial signal was *not* local, but rather the manifestation of a generally distributed electrical field.

In obtaining these cancellations no effort was made to use a reference terminal approximating the electrical heart center, since such a reference is entirely unnecessary for cancellation. In 9 of the 30 patients in which it was attempted, the location of the site of the mirror pattern was predictable from data obtained in human torso models.

It would seem reasonable to believe that a peculiar precordial pattern would be a local phenomenon, but when the identical peculiarity is consistently found at a distant and predictable point, the concept of a localized current generator producing localized precordial patterns must be re-examined.

Experimental Chronic Pyelonephritis in the Rat. ALVIN P. SHAPIRO, ABRAHAM I. BRAUDE, and JENNIE SIEMIENSKI, Dallas, Texas. (Introduced by Carleton B. Chapman).

It is widely accepted that chronic pyelonephritis leads both to hypertensive vascular disease and to an inflammatory destruction of the kidney that persists after infection disappears. Because this has been difficult to prove in human subjects, an effort has been made to develop an experimental model for studying chronic pyelonephritis.

In a previous report, we described a method for producing acute hematogenous pyelonephritis in rats by intravascular injection of *Escherichia coli* followed by massage of the kidneys. The resulting infection usually subsides within six weeks. To produce chronic pyelonephritis, therefore, rats were given four injections of *E. coli* during seven months. Periodically, urine obtained by cystotomy was examined and the BUN determined. Blood pressures (tail plethysmographic method) were measured every two weeks.

Twelve rats survived and were sacrificed after eleven months. Kidney cultures at autopsy revealed *E. coli* in only one animal, although earlier urine examinations had consistently disclosed bacteruria and pyuria. All, however, demonstrated typical features of chronic bilateral pyelonephritis. Kidneys were distorted, lobulated by scars, and differed in size, with thinning of cortex and thickening of pelvic walls. Interstitial inflammation composed of lymphocytes and plasma cells extended throughout cortex and medulla. Tubules were dilated, and contained colloid and "pus" casts. Connective tissue was increased markedly with periglomerular fibrosis, although glomeruli were uninvolved. Vascular lesions were absent, and blood pressures never became elevated. Despite extensive renal damage, sufficient function remained to prevent azotemia. Control rats, and those subjected to renal massage or bacterial inoculation alone, did not develop significant lesions.

It is concluded that 1) chronic bilateral pyelonephritis has been established experimentally; 2) persisting renal inflammation without infection was demonstrated; 3) the inflammatory reaction did not result directly in vascular disease or hypertension within the period of study. Development of hypertensive vascular disease may require additional disturbances or still greater renal destruction.

Body Composition in Normal and Abnormal States. WILLIAM SIRI and NATHANIEL I. BERLIN, Berkeley, Calif. (Introduced by John H. Lawrence).

The proportions of body fat, water and solids need broader investigation to evaluate the significance of alterations in body composition engendered by certain diseases and particularly the influence of fat in degenerative disease. Fat and lean body mass calculated from either total body water or body density normally are accurate but the method is invalid for abnormal hydration and perhaps obesity. The restriction is removed, however, if both water and density are determined. Using this procedure, gross body composition was investigated in several hundred healthy subjects and fifty patients with blood dyscrasias. Normal subjects included both sexes, ages 21 to 90, and body fat from 4 to 60 per cent. Total body water was measured with tritium. Body density was obtained by a new method developed especially for its toleration by aged and ill subjects; a helium dilution technique for which accurate corrections for thermal and respiratory gas effects were developed. Studies on healthy subjects were examined for variability of body composition in the adult population, for age trends in body constituents, and dependence of extracellular fluid on non-essential fat. Studies on patients were directed toward correlating changes in fat, water and lean tissue as an aid to differential diagnosis and management of disease. Concurrent blood volume studies with Cr^{51} and P^{32} labeled red cells on 30 of the normal subjects suggest a closer correlation with fat-free body weight than with total weight. Preliminary analysis gives a red cell volume of 36 ml. per Kgm. of lean body and 4 ml. per Kgm. of fat. Similar studies on 20 patients with polycythemia and leukemia show abnormal blood volumes to be somewhat better differentiated when expressed in percentage of lean body weight.

Metabolic Factors Influencing the Susceptibility of Mice to Staphylococcal Infection. J. MACLEAN SMITH and RENÉ J. DUBOS, New York, N. Y. (Introduced by Maclyn McCarty).

The course of infection induced in mice by intravenous injection of staphylococci has been studied by the quantitative determination of the fate of the cocci in the different organs and by the fatality rate of the animals. Using both methods of study it has been possible to show that host susceptibility can be increased by various metabolic disturbances.

The feeding of desiccated thyroid, thyroxin or 2:4 dinitrophenol increases the severity of a standard infection. This increase cannot be brought about by feeding di-iodo tyrosine or iodine. The cocci survive in the organs of thyroid treated mice for a longer period than in the control animals. The enhancing effect of thyroid extract on experimental infections obtains also with a number of other bacterial and mycotic pathogens.

Short periods of fasting also increase the susceptibility of mice to staphylococcal infection. A period of 36 hours is sufficient to cause increase in susceptibility. Resistance to infection becomes normal again after 24 hours of normal diet following a period of fasting. The administration *ad lib* of 5 per cent glucose solution as drinking fluid during fasting increases susceptibility. Administration of 1 per cent sodium lactate in a similar fashion corrects partially the infection enhancing effect of fasting.

These experimental findings may be of significance in the analysis of factors which interfere with treatment in certain staphylococcal infections in man.

Further Experiences with Serum Quinidine Concentration as a Guide in the Treatment of Chronic Auricular Fibrillation and Flutter. MAURICE SOKOLOW,* San Francisco, Calif.

Conversion to sinus rhythm was attempted 215 times in 179 patients with chronic auricular fibrillation or flutter. Normal rhythm was achieved in 52 per cent of the 94 cases with rheumatic heart disease, 80 per cent of the 85 non-rheumatic cases, and 71 per cent of the total group; successful conversion occurred at a mean serum concentration of 5.7 micrograms per ml.

Serum quinidine concentrations were determined frequently during the administration of progressively larger doses of the drug. In different individuals, a given daily dosage resulted in a wide range of serum levels, and often did not correlate well with myocardial or gastrointestinal toxicity. On the other hand, a good correlation was found when toxicity was related to serum concentrations, of which 650 were available for study. Toxicity severe enough to require cessation of the drug rose from 1 per cent for levels under 6 micrograms per ml., to 8 per cent, 16 per cent, 25 per cent and 38 per cent for levels from 7 to 10 micrograms per ml., respectively.

The daily dose necessary to effect conversion was 3 Gm. or less in 80 per cent in those cases which converted. On a dose schedule of 3 Gm. per day, peak serum concentrations in 59 instances averaged 7.4 micrograms per ml. but 37 per cent exceeded 8 micrograms per ml., and 20 per cent were less than 5 micrograms per ml. This variation of serum concentration must be appreciated because 77 per cent of the successful conversions occurred at levels below 8 micrograms per ml. while toxicity increased sharply when this level was exceeded. Ninety per cent of the conversions occurred at levels below 10 micrograms per ml.

The best clinical management, therefore, demands caution and a reconsideration of the indication for conversion with quinidine when the serum concentration reaches 8 micrograms per ml. and sinus rhythm has not been restored. Increasing the dose beyond this point leads to a sharp rise in the incidence of serious toxicity, while the chances of conversion are considerably less than they were at the beginning of treatment.

The Effect of Altered Thyroid Function on the Localization of Radioactively Labeled Pituitary Extracts in the Thyroid and Other Tissues. MARTIN SONENBERG and WILLIAM L. MONEY, New York, N. Y. (Introduced by Rulon W. Rawson).

A pituitary preparation rich in thyrotrophic and gonadotrophic activity has been labeled by dissolving the hormone preparation in S^{35}-labeled concentrated sulfuric acid at 0° C. The radioactive preparation was then diluted and the non-protein bound radioactivity was removed by dialysis against isotonic saline.

Such radioactive sulfur labeled pituitary preparations lost completely their capacity to increase the weight of chick thyroids. However, they did retain some of their original gonadotrophic activity. Following injection into rats of such radioactively labeled pituitary preparations, the greatest concentrations of radioactivity were found in the thyroid, testes, retro-orbital tissues, liver, spleen, and kidney. The concentration of radioactivity decreased from the initial maximum at 5 minutes. At 24 hours the concentration of radioactivity in the thyroid, retro-orbital tissues, and testes were 45, 12, and 8 times greater than that found in blood, respectively. Control injections containing S^{35}-labeled inorganic sulfate and an unlabeled pituitary preparation showed insignificant concentrations of radioactivity in the thyroid, retro-orbital tissues, and testes.

Thyroid function was altered to determine the effects on the localization of radioactivity after administration of similarly labeled pituitary hormone preparations. The effects of surgical thyroidectomy, treatment with thiouracil or iodine-poor diet or thyroxine or large doses of sodium iodide were included in this study. The administration of the labeled hormone preparation to animals on an iodine-poor diet resulted in an increased localization of radioactivity in the thyroid. There was increased localization of radioactivity in the retro-orbital tissues of animals maintained on an iodine-deficient diet or on thiouracil. Other methods of altering thyroid function failed to influence the localization of radioactivity after the administration of S^{35}-labeled pituitary preparations.

Thromboplastin Inactivation in Human Blood. THEODORE H. SPAET, San Francisco, Calif. (Introduced by Paul M. Aggeler).

It has been demonstrated that human blood generates a potent thromboplastic activity during clotting. Since this activity is not present in stored serum, a mechanism must be available for its removal. In the present study the basic technique employed was derived from the thromboplastin generation test of Biggs and Douglas. When this test is performed with diluted reagents as originally described, the formation of thromboplastic activity reaches a peak within two minutes, and there is little reduction of activity even after one hour of incubation. If the plasma and serum reagents are used undiluted, thromboplastin generation is normal, but appreciable loss of activity occurs within 14 minutes. These findings suggest an active removal mechanism rather than intrinsically unstable thromboplastic activity.

An assay method has been devised to measure the ability of a specimen to inactivate blood thromboplastic activity. The unknown specimen is mixed with sodium citrate, and added to the thromboplastin generating mixture five minutes after the beginning of the test. The resulting mixture is tested for thromboplastic activity after an additional 30 minutes of incubation. Both plasma and serum inactivate thromboplastin, and their ability to do this is not reduced by heating to 56° C. for 30 minutes, by barium sulfate adsorption, or by ether extraction. Of the Cohn fractions, only fraction V inactivates thromboplastin. However, hypoalbuminemic sera behave similarly to normal serum. Sera of patients with hemophilia and PTC deficiency have no increased ability to reduce thromboplastic activity.

Intestinal Secretion, Absorption and Excretion of Cholesterol in Man. MALCOLM M. STANLEY* and SAMUEL H. CHENG, Boston, Mass.

A special diet, chiefly skim milk solids with added vegetable fat (60 mg. cholesterol, 101 gm. fat daily), allowed cholesterol to be excreted unchanged in the stools. Conversion to other digitonin-precipitable sterols in the intestine was avoided. Stool cholesterol excretion was estimated by a chromic oxide inert indicator method. Cholesterol secretion into the gut was estimated from dilution of ingested radioactive cholesterol. Absorption was calculated either from intake plus secretion minus excretion, or by application of the inert indicator method to the excreted radioactive cholesterol.

Three healthy men, 29 to 38 years old (fasting serum cholesterols *163 to 239* mg. per cent, *72 to 74* per cent esters), secreted 1.9 to 2.2 gm., absorbed 1.5 to 1.8 gm., excreted 0.35 to 0.64 gm., daily. Other subjects had values expressed as above: (*1*) F, 47, *carcinoma head pancreas, complete biliary obstruction* (*375* mg. per cent, *18* per cent esters), 0.17, 0.08, 0.14; (*2*) M, 43, *familial xanthomatosis, diabetes mellitus, coronary disease, recent high-fat diet treatment duodenal ulcer* (*425* mg. per cent, *71* per cent esters), 3.1, 2.7, 0.47; (*3*) M, 40, *non-tropical sprue* (*119* mg. per cent, *70* per cent esters), 0.26, 0.15, 0.17; (*4*) M, 21, *chronic active regional enteritis, shortened small intestine, jejuno-transverse colostomy, severe malnutrition* (*60* mg. per cent, *65* per cent esters), 0.72, 0.34, 0.44; (*5*) M, 67, *idiopathic hyperlipemia* (*387* mg. per cent, *64* per cent esters), 1.5, 1.2, 0.37; (*6*) F, 62, *chronic cholangiolitic hepatitis, slight regurgitation jaundice* (*450* mg. per cent, *68* per cent esters), 2.1, 1.4, 0.74.

With extremely low intake most cholesterol measured was endogenous. Intestinal exchange of endogenous cholesterol is a very active process which involves daily amounts equivalent to a significant portion of total readily exchangeable body cholesterol. Considerable variations occur in some diseases of cholesterol metabolism. Measures which can appropriately influence one or more phases of this process are logical in treatment of some of these disorders.

Physiologic and Therapeutic Effects of the Intravenous Administration of Streptococcal Fibrinolysin-Activating Principle (Streptokinase) in Man. MARIO STEFANINI* and LUCY SALOMON, Boston, Mass.

Following their partial purification, Streptokinase-Streptodornase (Varidase®) preparations have been injected intravenously in humans (Federation Proc., 1954, 13, 125). Doses up to 10,000 units per kilo weight were administered over a 1 to 4-hour period, with absent or mild febrile reaction and moderate hypotensive effect. Injection was followed by increased plasma fibrinolytic activity; lowered plasma fibrinogen, profibrinolysin and antifibrinolysin level for a period of 30 minutes to 12 hours following the infusion. Changes in platelets, prothrombin and labile factor levels were variable, and usually moderate. Bleeding tendency (oozing at the site of venous or finger puncture) was often noted at the height of plasma lytic activity. These results have recently been confirmed and extended by Tillett *et al.* (J.C.I., 1955, 34, 169).

Forty-nine normals and patients have received purified Varidase® intravenously during the past four years. Fourteen constituted the original "physiologic" series. Additional 35 patients were given Varidase® intravenously in the attempt of determining lysis of the intravascular clot. Thirty were suffering from deep thrombophlebitis, 3 with coronary thrombosis, 1 with mesenteric thrombosis and one with thrombosis of the right central retinal vein. They received purified Varidase® for a total dose of 1,000 units per kilo weight per hour: (a) as continuous infusion for four hours, or (b) in two separate infusions of two hours' duration each, with a 6-hour intervening period.

Previously reported changes in the hemostatic mechanism were confirmed. The following clinical response was observed: (a) prompt, considerable decrease of inflammatory reaction, with reduction of pain and edema in patients with deep thrombophlebitis; (b) patient with mesenteric thrombosis recovered; partial success was obtained in the case of thrombosis of the central retinal vein; no appreciable effect was observed in coronary thrombosis, possibly, some reduction in pain; (c) embolic complications (pulmonary infarction) occurred in one patient. Clinical and experimental results will be presented in detail.

Rate of Peripheral Utilization of Thyroid Hormone in Hypermetabolism without Primary Endocrine Disease. KENNETH STERLING,* and ROBERT B. CHODOS, Syracuse, N. Y.

Established clinical precept recognizes that hypermetabolism associated with leukemia, hyperpyrexia and pulmonary disorders may, in some instances, simulate Graves' Disease despite absence of evident primary pathology of the thyroid gland.

The rate of disappearance of intravenously injected I-131 labeled l-thyroxine has been employed to measure the rate of peripheral degradation or utilization of thyroid hormone. Normal volunteers degraded 45 to 60 micrograms of organic iodine daily; myxedematous patients, 10 to 20 micrograms; and typical cases of Graves' Disease, partially controlled on anti-thyroid drugs and/or iodine therapy, degraded 100 to 200 micrograms per day.

Intravenous injections of 1 mg. of l-thyroxine in myxedematous patients failed to alter the slope of the radiothyroxine disappearance curve, prior to the onset of the calorigenic effect.

Patients with leukemia or fever were selected for study as euthyroid subjects with peripheral hypermetabolism, on the basis of clinical appearance and high basal metabolic rate, but normal protein-bound iodine concentration. Such patients degraded approximately 100 micrograms of iodine per day, a figure in excess of the normal range.

These phenomena suggested a secondary or compensatory hyperactivity of the thyroid gland in response to increased metabolic activity in the peripheral tissues. It is inferred that peripheral tissue metabolism has an important role in determining the hormone degradation rate.

The Correlation Between Serum Potassium and the Weight of the Adrenal Glomerulosa in Rats. H. C. STOERK, A. I. KNOWLTON,* and E. N. LOEB, New York, N. Y. and Rahway, N. J.

Circumstantial evidence suggests that the adrenal glomerulosa is concerned with the elaboration of Aldosterone. Earlier studies have indicated that changes in the glomerulosa occur with variations in sodium intake. In this study the relationship of serum Na and K concentrations to glomerulosa weight was studied in rats fed a wide range of Na and K. Nine combinations of three dietary levels of Na (0.001, 0.05, 1.0 per cent) and K (0.001, 0.16, 0.7 per cent) were fed for two weeks to nine groups of six rats. Nine identical groups were injected with 2 mg. of DCA daily. At sacrifice serum Na and K were determined with the flame photometer, the adrenals weighed and serially sectioned. Planimeter measurements of enlarged projections of the glomerular zone served for the calculation of its weight.

None of the nine combinations of dietary Na and K caused appreciable changes in serum Na concentrations. However, there was marked hypokalemia in the groups with low and hyperkalemia in those with high K intake. At all levels of dietary K an increase in dietary Na caused a depression of serum K. Since no differences obtained between the DCA treated and untreated groups they were combined for evaluation. From 36 serum K determinations, each derived from the pooled blood of three rats, and from 36 average weights of the glomerulosa in the same animals, a direct relationship was found between the weight of the glomerulosa zone and the level of serum K. The glomerulosa weights ranged from 3.0 to 15.8 mg., serum K levels from 3.0 to 7.8 mEq. per L. The correlation was highly significant ($r = 0.6$, $p = < 0.01$).

Reconciliation of Theories of Freezing-Thawing Hemolysis of Erythrocytes. TIMOTHY R. TALBOT, JR. and STANLEY C. GLAUSER, Philadelphia, Pa. (Introduced by Thomas Fitz-Hugh, Jr.).

Lovelock's theory states that freezing-thawing hemolysis is the result of high salt concentration caused by

separation of pure ice from solution. Another theory suggests that freezing-thawing hemolysis results principally from the formation of ice crystals outside the erythrocyte, thus causing mechanical disruption.

We have found support for Lovelock's theory from temperature-time curves which were recorded for sucrose solution, saline, plasma, and erythrocytes. These curves showed fractional freezing most marked when erythrocytes were present. This theory predicts that the lower the temperature, the greater the salt concentration and therefore the greater the hemolysis. Hemolysis would be zero at 0° C. and increase with decreasing temperature until −50° C. where there would be 100 per cent hemolysis. Any temperature below −50° should lead to 100 per cent hemolysis according to this theory.

The second theory finds support in our hands from: (a) the fact that materials which prevent hemolysis at −79° C. strongly bond hydrogen and distort the ice lattice; (b) our data showing that high molecular weight polyoxy compounds prevent hemolysis due to freezing at −79° although these compounds do not enter erythrocytes. This theory also predicts that there should be a sharp increase in hemolysis as the temperature goes from −60° C. to −45° C., and that from −45° C. to 0° C. there should be 100 per cent hemolysis.

Curves of hemolysis *versus* temperature show a maximum in the region −45° C. to −55° C. When temperatures from −50° C. to −192° C. are used the range from 0° C. to −40° C. is passed rapidly and the salt effect is small compared to the effect of ice crystals. However, from 0° C. to −40° C. the effect of ice crystals is small compared to the effect of salt.

It is therefore suggested that both mechanisms are operative, with damage due to high salt concentration predominating from 0° C. to −50° C. and disruption due to ice crystals predominating at lower temperatures.

Selective Inhibition of Virus Multiplication by β-D-Ribofuranosides of Benzimidazoles. IGOR TAMM, New York, N. Y. (Introduced by Frank L. Horsfall, Jr.).

Virus multiplication clearly depends on the metabolism of the infected cell. However, it is now evident that the biosynthetic demands of the virus are not identical with those of the cell. One area of probable difference is nucleic acid synthesis. This may be approached through inhibition by chemical compounds of known structure.

The benzimidazole derivatives used were made available by Dr. Karl Folkers of Merck and Company, Inc., and were synthesized with the idea that they might affect nucleic acid metabolism. Certain of these compounds inhibit markedly the multiplication of influenza A and B viruses, and of mumps and vaccinia viruses in various host tissues. They act after the virus has entered the cell but before new virus particles appear.

The very high inhibitory activity of di- and trichlororibofuranosyl compounds on influenza virus multiplication is dependent on: 1) β-D-ribofuranose at N1 in the imidazole ring; 2) multiple chlorine substituents in the benzenoid ring; activity increases as the number of chlorine atoms is increased.

The 5,6-dichloro-1-β-D-ribofuranosyl derivative inhibits influenza virus synthesis more than the metabolic activities of host tissue. Its inhibitory effect on influenza virus is greater than that on vaccinia virus. Selectivity of action of both kinds is dependent on β-D-ribofuranose in the imidazole ring. Any departure from this structure causes lowering of both influenza virus inhibitory activity and selectivity of action. The pentose moiety of the inhibitor molecule is thus identical with that in ribonucleic acid of host cells and with that in the nucleic acid of the influenza virus particle, but different from the β-D-desoxyribose moiety in the nucleic acid of vaccinia virus.

The findings show that with certain compounds virus inhibitory activity and toxicity vary independently. Through appropriate modification of the structure, virus inhibitory activity can be increased without increasing tissue toxicity to the same extent.

A Comparison of Electrocardiographic, Electroencephalographic, and Chemical Effects of Rubidium and Potassium in Dogs. R. TARAIL, T. E. BENNETT, and W. K. NOELL, Buffalo, N. Y. (Introduced by David K. Miller).

Discrepancies exist among chemical, clinical, and electrocardiographic phenomena of potassium intoxication in man. Therefore other metallic ions which simulate potassium intoxication were sought. Rubidium was chosen because of physiologic and chemical similarities.

Isotonic solutions of either rubidium (six dogs) or potassium chloride were injected intravenously into ten pentobarbitalized dogs (9.4 to 16.0 kg.) at rates between 0.49 and 0.70 ml. per kg. per minute. The mean lethal dose of rubidium appeared significantly greater. Both ions produced cardiotoxic death. The electroencephalogram was unchanged until terminally when attenuation and discontinuity suddenly supervened. Respiratory and electroencephalographic arrest were invariably simultaneous, occurring between 29 and 60 minutes, and antedated electrocardiographic cessation. The classical terminal electrocardiographic events of potassium intoxication (intraventricular block and bradycardia) also resulted from rubidium but were overshadowed by prolonged ventricular tachycardia-ventricular flutter and by intraventricular block with less bradycardia.

The earliest electrocardiographic correlate of potassium poisoning was peaking and increased inversion or uprightness of T waves (Lead II) within 2 to 12 minutes at increments of serum potassium of 0.7 to 4.1 milliequivalents per liter above control values. Fading of the P wave, auriculoventricular and intraventricular blocks followed. Rubidium induced analogous changes and long portions of electrocardiograms were indistinguishable from those of potassium intoxication. But within 10 to 23 minutes ventricular premature systoles developed, and later characteristically alternated with non-premature beats, eventuating in long episodes of ventricular tachycardia and ventricular flutter in contrast to the much less significant role of ventricular hyperirritability after potassium administration.

Elevations in serum potassium proceeded in apparently linear fashion during potassium infusions to terminal concentrations of 16.7 to 18.0 milliequivalents per liter.

Both rubidium and potassium invariably provoked impressive reciprocal diminution of serum sodium.

Rubidium poisoning may engagingly mimic potassium but distinctive differences are evident.

The Production of Localized Necrotizing Angiitis by the Combined Effects of a Synthetic Acid Polymer and Endotoxin. LEWIS THOMAS,* New York, N. Y.

In previous investigations it was observed that the endotoxins of gram negative bacteria act synergistically with three heparin-like synthetic acid polymers, sodium polyanethol sulfonate (Liquoid®), sodium polyvinyl alcohol sulfonate, and dextran sulfate. Simultaneous intravenous injections of endotoxin and acid polymer, in amounts which have no demonstrable effect when given singly, cause widespread occlusion of small blood vessels by fibrinoid material, resulting in bilateral cortical necrosis of the kidneys, fibrinoid necrosis of the coronary arteries, and other lesions resembling those of the generalized Shwartzman reaction. During the period when intravascular fibrinoid deposition is occurring, the plasma fibrinogen falls to very low levels. In heparinized animals, fibrinoid does not appear, no diminution of fibrinogen occurs, and no lesions develop in the kidneys or other organs.

In order to explore the possibility that fibrinoid may consist of precipitated fibrinogen in combination with acid polymer, the local effect of Liquoid® in skin tissue was studied in animals simultaneously receiving endotoxin by vein. Within 24 hours after injection, nodules appeared at the site of Liquoid® injection. Throughout the nodules, the small and medium-sized arteries showed severe degrees of polyarteritis, with infiltration of all coats of the wall by polymorphonuclear leucocytes. Dense masses of homogeneous eosinophilic material resembling fibrinoid appeared within the arterial walls, and pools of similar material surrounded the arteries. After 3 or 4 days, the cellular infiltrate in the vessel walls became predominantly mononuclear, with large masses of cells occupying the intima. After 10 days, the lesion became converted to a granulomatous type of arteritis, with numerous multinucleated giant cells.

The observations indicate that polyarteritis nodosa is associated with deposition of precipitated fibrinogen in and around the walls of arteries, due to the presence of an acid polymer in the surrounding tissue. A possible mechanism which may lead to increased precipitability of fibrinogen in animals treated with endotoxin, demonstrable by cold-precipitation of the fibrinogen by heparin, will be discussed.

The Relationship of Serum Levels of Calcium and Phosphate to Parathyroid Function in Man. DAVID D. THOMPSON and HOWARD H. HIATT, Bethesda, Md. (Introduced by Robert F. Pitts).

A precise definition of the role of calcium and phosphate in the regulation of parathyroid function has been hindered by the fact that a change in the concentration of either of these ions in the body fluids usually leads to an alteration in the concentration of the other.

We have demonstrated that renal phosphate reabsorption can be correlated with the amount of parathyroid extract administered. Therefore, if variations in serum calcium or phosphate were to affect parathyroid function, an alteration of phosphate Tm might be expected.

In our studies the inulin clearance was measured and buffered phosphate was infused in sufficient quantity to saturate the renal tubular mechanism for phosphate reabsorption (phosphate Tm).

In normal subjects phosphate Tm was maximal in association with a depressed serum phosphate produced by prolonged administration of aluminum hydroxide gel. This effect was independent of the calcium intake. Reduction of serum phosphate in a hypoparathyroid individual did not alter phosphate Tm.

In a normal subject five successive daily phosphate infusions resulted in a decrease in phosphate Tm from 102 micromols per min. to 0 micromols per min. In contrast, two hypoparathyroid individuals showed minimal changes in phosphate Tm on similar regimens. To ascertain whether the fall in serum calcium which accompanied the elevation in serum phosphate was responsible for the observed change in Tm, disodium calcium ethylenediamine tetraacetic acid (EDTA) was administered intravenously for five days. Reductions in serum calcium comparable to those seen with phosphate infusions resulted. However, no rise in serum phosphate occurred and there was no change in phosphate Tm. Elevations of serum calcium produced by infusions of calcium gluconate also failed to alter phosphate Tm.

The data suggest that variations of serum inorganic phosphate rather than of serum calcium regulate the secretion of a parathyroid hormone affecting renal phosphate reabsorption.

The Effect of Pantothenic Acid Deficiency on Gastric Secretion and Motility. G. H. M. THORNTON, W. B. BEAN,* and R. E. HODGES, Iowa City, Ia.

A study of gastric function was carried out on three human volunteers in whom pantothenic acid deficiency was induced by simultaneous administration of a synthetic deficient diet and a metabolic antagonist to panthothenic acid. The response to histamine, hypoglycemia and a modification of the serial test meal (Hunt, J. N., and Spurrell, W. W.: J. Physiol., 1951, **113**, 151) were used to measure the secretory function of the stomach. The test meal data were also used to determine the effect of the experiment on gastric motility. These tests were carried out before and during the development of deficiency and were repeated after recovery.

The secretory response in each test was expressed as volumes of parietal and non-parietal components of gastric juice in milliliters and total quantity of pepsin in Anson units. In two of the three subjects the development of deficiency was associated with a marked fall in all constituents of gastric juice except the pepsin response to the test meal. Restoration of function during the final control period was complete. The gastric emptying pattern of each subject remained the same throughout the experimental and control periods.

Pantothenic acid deficiency is believed to have interfered with the metabolism of the parietal cells. This

may have been an indirect effect of diminished secretion of adrenal cortical hormones, or may indicate that Coenzyme A is essential to the mechanism of hydrochloric acid secretion by the parietal cells.

The Effect of a Low Sodium Diet and the Effect of Nor-epinephrine on the Electrolyte Composition of Arterial Wall. Louis Tobian, Jr.,* Minneapolis, Minn.

Normal rats on a diet containing .3 per cent sodium were compared to similar rats on a diet containing .003 per cent sodium. The "low sodium" rats had 32.6 mEq. of sodium per 100 gm. of aorta solids compared to 37.0 mEq. in the "high sodium" rats, a highly significant difference. While the sodium content of the aorta decreased 12 per cent after a low sodium diet, the chloride content was practically unchanged. Moreover, sodium and chloride concentrations in serum were similar in the two groups. Sodium lost from the aorta during the low sodium diet is mainly intracellular. The low sodium diet also produces a significant rise in the intracellular phosphorus content of aorta, but no change in potassium. Changes in artery composition may account for the protective action of low sodium diets against various forms of hypertension.

In rats with renal hypertension the aorta has a significantly elevated potassium and phosphorus content. When a low sodium diet lowers the elevated blood pressure to normal, the aorta potassium and phosphorus also revert significantly toward normal.

Fourteen dogs infused with nor-epinephrine in saline were compared with five saline controls. One femoral artery was obtained before each infusion; the other during the infusion. Nor-epinephrine produced a highly significant fall in the artery potassium content, the decrease varying from 4 per cent to 59 per cent (average 27 per cent). The controls showed an average increase of 17 per cent in artery potassium content. Nor-epinephrine also produced a significant increase in artery sodium content, an average gain of 1.7 mEq. per 100 gm. solids, while the controls were losing 0.4 mEq. However, five "nor-epinephrine" dogs scarcely gained any artery sodium while losing considerable artery potassium. The mEq. gain in sodium after nor-epinephrine averaged only ¾ the loss of potassium. Nor-epinephrine didn't significantly change the water and chloride in arteries.

Enzymatic Metabolism of Steroids. Gordon M. Tomkins, Bethesda, Md. (Introduced by Henry Barnett).

The major metabolic fate of the steroid hormones consists of, first, hydrogenation of the double bond of ring A to form the dihydro compound, followed by reduction of the 3-keto group to give the inactive 3-alcohol which is then excreted in the urine. In this manner, cortisone is first reduced to dihydrocortisone which is further reduced to tetrahydro cortisone.

In order to investigate these reactions in greater detail, purified enzyme preparations from mammalian liver have been obtained which are capable of carrying out the reductive metabolism of a wide variety of steroids in a stepwise manner. In the initial attack on the steroid nucleus, hydrogenation of the double bond of ring A, the hydrogen donor is either reduced di- or triphosphopyridine nucleotide (DPNH or TPNH) depending on which steroid is serving as the substrate. The enzymes catalyzing this reaction are highly specific. Thus evidence based on enzyme purification procedures suggests that separate enzymes are involved in the reduction of the androgens, the adrenal steroids, and cholesterone, and that even within one class (*e.g.*, the corticoids), different compounds are metabolized by separate enzymes.

The second reaction under investigation concerns the reduction of the 3-keto group of androgenic and cortical steroids with the formation of the corresponding 3-alcohol. In the second reaction, the reduction of the keto group, in contrast to the preceding one, all the steroid substrates are metabolized by a single enzyme, and either DPNH or TPNH can serve equally well as a hydrogen donor.

The studies reported here provide the basis for a sensitive, specific enzymatic assay for a considerable variety of steroids.

Flexion Deformities in Adrenal Insufficiency. W. P. Vander Laan, Boston, Mass. (Introduced by Joseph Hayman, Jr.).

Flexion deformities of the extremities ("tendon contractures") have been observed in Addison's disease treated with desoxycorticosterone. It has been considered a toxic manifestation of treatment. Another interpretation is offered based on observations in three cases in which flexion deformities were noted prior to treatment.

A 41-year-old woman had typical Addison's disease and "tightness" in the thighs with inability to straighten the legs or hips. Leg motion was painless within 20° of flexion but extension beyond this caused pain. Traction resulted in inflammation of the knees and fever. The deformity grew more severe although normal electrolyte levels were maintained. No abnormality of joint or muscle was established by x-rays, electromyogram, muscle biopsy, or creatine excretion levels. Finally a 90° flexion deformity of the legs was noted. Desoxycorticosterone was stopped when cortisone became available; with 10 mg. daily doses improvement occurred. Prior to death from influenza she had recovered to her original state. Autopsy disclosed adrenal atrophy, moderate atrophy of hamstring muscles, no joint disease, and normal tendons and sciatic nerves.

A 75-year-old woman had "arthritis" of legs of 9 years' duration. The legs and hips were held in extreme flexion. This could be overcome with extreme difficulty. Hypoglycemia and hypochloremia were noted. Autopsy disclosed pituitary destruction and no abnormality of the legs.

A 57-year-old woman had an enlarged sella turcica and evidence of hypoadrenalism. The legs were kept at 25° flexion; further extension caused severe pain. With 25 mg. cortisone daily the legs became normal.

These observations suggest flexion deformities occur spontaneously in adrenal atrophy and pituitary insufficiency, probably because of hydrocortisone deficiency.

It is likely that desoxycorticosterone, and perhaps aldosterone, aggravate this condition. Muscle spasm, not "tendon contractures," is observed.

Serum Cholesterol Esterase (Sperry Enzyme) in Idiopathic Hypercholesteremia, Idiopathic Hyperlipemia, and in Coronary Artery Disease. CHUN-I WANG and DAVID ADLERSBERG, New York, N. Y. (Introduced by Milton Mendlowitz).

The activity of serum cholesterol esterase (Sperry Enzyme) was studied in four groups of patients: (I) 14 normocholesteremic controls, (II) 33 patients with idiopathic hypercholesteremia, (III) 10 with idiopathic hyperlipemia and (IV) 14 normocholesteremic patients with proven coronary artery disease.

The average values were as follows: Group I, total serum cholesterol 225 ± 35, free cholesterol 59 ± 11 mg. per cent, cholesterol esterase 41 ± 10 units; Group II, total cholesterol 372 ± 92, free cholesterol 101 ± 31 mg. per cent, cholesterol esterase 26 ± 11 units; Group III, total cholesterol 533 ± 148, free cholesterol 181 ± 48 mg. per cent, cholesterol esterase 25 ± 8 units; Group IV, total cholesterol 204 ± 35, free cholesterol 54 ± 10 mg. per cent, cholesterol esterase 32 ± 8 units. There was no straight relationship between the activity of cholesterol esterase and the levels of free serum cholesterol. None of the patients presented evidence of hepatic damage.

The difference in cholesterol esterase activity between groups I and IV was significant at the 0.03 level, that between groups I + IV and groups II + III was significant at the 0.0001 level. Serum cholesterol esterase activity was diminished in groups II, III and IV although the ratio between free and total serum cholesterol was normal (approximately 30 per cent). Difference in esterification of serum cholesterol in the various errors of lipid metabolism was suggested in previous studies performed with labelled cholesterol and acetate (J. Clin. Invest., **34**: 48, 1955).

Agewise Standard Value for C_{In}, C_{PAH}, and Tm_{PAH} in Adult Males. DONALD M. WATKIN and NATHAN W. SHOCK, Bethesda and Baltimore, Md. (Introduced by Charles G. Zubrod).

Standard values per 1.73 M^2 surface area for inulin clearance and for effective renal plasma flow and Tm as determined with Diodrast® have been reported in a series of 70 subjects by Davies and Shock. In the present study, C_{In}, C_{PAH}, and Tm_{PAH} were measured under basal conditions in 110 male subjects ranging in age from 19 to 92 years. From each of six age categories subjects were selected for study on the basis of history, physical findings, and urine analysis. All were free from manifest renal disease, diastolic hypertension or myocardial failure. All were ambulatory, cooperative, and afebrile.

Standard C_{In}, C_{PAH} and Tm_{PAH} declined in a linear fashion with age from the fourth through the ninth decades. Filtration fraction rose significantly, C_{In}/Tm_{PAH} showed no significant change, and C_{PAH}/Tm_{PAH} declined significantly with age.

The following regression equations (X = age in years) were computed by the method of least squares for the functions measured per 1.73 M^2 surface area and the ratios derived:

$$C_{In} = -1.163X + 157.0\ *$$
$$C_{PAH} = -6.753X + 820.2\ *$$
$$Tm_{PAH} = -.8648X + 120.6\ *$$
$$ff. = +.0715X + 17.51\ *$$
$$C_{In}/Tm_{PAH} = -.00158X + 1.382$$
$$C_{PAH}/Tm_{PAH} = -.0278X + 7.710\ *$$

* Regression significant, $P < .001$

Approximations of standard values for age may be quickly computed by assuming a decrement of one ml. per min. per year of age from a level of 130 ml. per min. at age 30 for C_{In} and one mg. per min. per year of age from a level of 100 mg. per min. at age 30 for Tm_{PAH}.

The Production of Impending Hepatic Coma in Alcoholics with Cirrhosis by a Carbonic Anhydrase Inhibitor (Diamox®). LESLIE T. WEBSTER, JR., Boston, Mass. (Introduced by Charles S. Davidson).

Impending hepatic coma, characterized by confusion and the typical "flapping" tremor, previously observed in cirrhotic patients given oral nitrogenous substances—ammonium exchange resins, ammonium salts, urea, and increments of dietary protein—is here described following oral administration of a carbonic anhydrase inhibitor, Diamox® ($C_4H_6N_4O_3S_2$).

Of twelve alcoholics with cirrhosis given from 500 mg. of Diamox® on alternate days to 1000 mg. daily for periods varying from three to twenty-seven days, four developed mild to marked confusion (one during three separate periods). One had the "flapping" tremor previously; two developed it, one during three separate periods. All four had longstanding severe liver disease and became confused within forty-eight hours of the initial dose (two after 500 mg.). Three had a history of confusion or coma related to liver disease. Measurements of venous blood "ammonia" concentrations showed increases to abnormal levels during three of four periods. Serum potassium decreased during five of six periods, significantly during three. Potassium chloride orally, during three periods, did not prevent or alleviate confusion although serum potassium concentrations remained normal or dropped slightly.

Of the eight remaining patients, without history of confusion or coma, none developed confusion or tremor; three developed drowsiness. Five had liver disease comparable to that of the group developing confusion. One developed a slightly elevated blood "ammonia" concentration of questionable significance; one, a low serum potassium concentration. Most of the twelve patients observed showed a rise in serum carbon dioxide and chloride concentrations but no significant change in sodium.

Five hundred milligrams of Diamox® produced the syndrome of impending hepatic coma in susceptible patients with cirrhosis, but presumably by a different mechanism from that of the previously implicated nitrogenous substances because it contains little or no available nitrogen. Potassium chloride neither prevented nor improved

the syndrome although decreased serum potassium concentrations often occurred.

The Mechanism of Cardiac Failure in Tachycardia. RENÉ WÉGRIA,* H. H. WANG, and V. V. GLAVIANO, New York City, N. Y.

In dogs anesthetized with chloralose, left ventricular output and coronary sinus outflow were continuously measured with rotameters and mean arterial blood pressure continuously recorded, the oxygen contents of arterial and coronary sinus bloods were determined at appropriate intervals. Auricular tachycardia of progressively increasing rates was induced by electrical stimulation of the left auricular appendage. It was observed that within the range of rates studied, each increment of heart rate resulted in a decrease of cardiac output and arterial blood pressure, an increase in coronary blood flow and oxygen consumption, and a decrease in cardiac work and efficiency. Whether the decrease in cardiac efficiency is caused by a relative decrease in coronary flow, which seems improbable, or whether it is due to the direct effect of the tachycardia on the myocardium, will be discussed.

The Role of Connective Tissue in Body Fluid Physiology. WILLIAM B. WEIL, JR. and WILLIAM M. WALLACE,* Cleveland, O.

Extracellular fluid (E.C.F.) electrolyte is, by generally accepted definition, in diffusion equilibrium with plasma. Such is not considered to be the case for intracellular fluid (I.C.F.) electrolyte. The present study was designed to determine, by direct analysis, the nature of the diffusion equilibrium between plasma and connective tissue.

In vitro diffusion experiments on rabbit skin and tendon, as examples of loose and dense connective tissue, indicate that the diffusible sodium and chloride of connective tissue are distributed in about 90 per cent of the tissue water, that less than 10 mEq. per kg. of each ion may be intracellular or bound to a tissue protein, and that in the connective tissue of the normal rabbit, as in other animals studied, the volume of distribution of chloride is greater than that of sodium.

In rabbits with a 20 per cent weight loss due to simple water deprivation, water and its contained electrolyte were lost in such a manner that usual ion ratios for connective tissue were maintained.

Adrenal insufficiency produced the expected increase in E.C.F. potassium and a decrease in E.C.F. sodium concentration but no change in chloride concentration. The connective tissue showed a relatively greater loss of chloride than sodium with consequent reversal of the usual sodium to chloride relationship. It can be inferred that water and sodium shifted "intracellularly." A similar reversal was noted in the connective tissue of two infants who died of severe hypertonic dehydration and in whom plasma chloride was elevated more than was the sodium concentration.

These data support the concept that connective tissue fluid and electrolyte are primarily extracellular in nature, but they also indicate that the equilibrium with plasma is not always one of simple diffusion and that only to a very limited degree can the connective tissue be a reservoir for "extra" anion or cation.

The Pathogenesis of Acute Peripheral Arterial Occlusion. STANFORD WESSLER and MARVIN GILBERT, Boston, Mass. (Introduced by Herrman L. Blumgart).

Some of the contradictory therapy recommended for the management of acute peripheral arterial occlusion rests on inadequate knowledge of the underlying pathologic physiology.

Utilizing SPCA-rich serum fractions and vascular stasis, an experimental method was developed in dogs for the routine induction of non-adherent femoral artery thromboses of known size and age which could be released to the periphery. Postmortem arteriography with radiopaque media that filled the arterial tree without crossing the capillary or venous beds, facilitated localization of the emboli and evaluation of the collateral circulation.

These studies have demonstrated that fresh 4 cm. clots within minutes after their release are routinely broken into multiple fragments at arterial bifurcations and after 72 hours are so reduced in size (lysis) that they cannot be recovered in the visualized arterial tree. A demonstrable preformed interarterial network 40 to 500μ in diameter effectively bypasses these emboli.

A similar injection study of human legs has shown that although fresh occlusions are present in one-half of extremities amputated for gangrene, they rarely produce severe ischemia in the absence of old occlusive disease. Without previous obstruction, fresh clots less than 25 cm. in length have not caused gangrene. Shorter occlusions are compatible with viability because the preformed collateral circulation provides an adequate bypass: hypertrophy of these anastomatic vessels can subsequently progress to permit return of pulsatile flow distal to an occlusion.

The fragmentation, dissolution, and ultimate length of the fresh arterial clot, the extent of prior occlusion, the response of the collateral circulation to the residual obstruction and systemic factors affecting ischemia (shock, anemia) can readily explain, without invoking the concept of "spasm," the phenomena associated with acute arterial obstruction. These observations permit formulation of a rational program for the management of acute peripheral arterial occlusion.

Thrombocytosis. K. J. R. WIGHTMAN, Toronto, Ont. (Introduced by R. F. Farquharson).

Patients with an excessive number of platelets may present various clinical pictures. This fact, together with the difficulty of doing platelet counts, seems to have obscured the diagnosis in many cases, and made the condition appear less common than it actually is.

A group of eleven such patients has been studied in the past few years. The manifestations of their disorder may be grouped as follows:

Thrombotic and embolic,
Hemorrhagic,
Constitutional and hematologic.

The severity of symptoms has ranged from mild to extremely grave. Two of the patients have died. Treatment of several cases with radio-active phosphorus has given generally satisfactory results, although the severest cases have been resistant to some extent.

The condition may be apparently primary, or secondary to a wide variety of diseases. The primary cases appear to have a natural history very like polycythemia vera, with a slightly elevated blood volume and a tendency to be associated with such conditions as hypertension, peptic ulcer, and Raynaud's phenomenon. However, frank polycythemia has not developed over a prolonged period of observation in any of these cases. A slight leukocytosis is not uncommon. In two cases the condition has developed after splenectomy, and this operation is contraindicated in persons who show evidence of the disease. Splenomegaly has been present in about half the patients. The marrow shows an increased number of megakaryocytes.

The bleeding which occurs resembles that seen in hemophilia or other defects of the coagulation mechanism. We have found no evidence to substantiate the view that the platelets lose their functional capacity as they increase in numbers. The exact mechanism of the hemorrhage has not been elucidated.

The condition probably occupies a position in the group of so-called myeloproliferative disorders, but in view of its distinctive features, deserves consideration as a separate entity.

Left Atrial Pressure Pulse Waves in Patients with Predominant Mitral Incompetence From Healed Rheumatic Endocarditis. JOHN B. WILD, GEORGE N. BEDELL, and JOHANN L. EHRENHAFT, Iowa City, Ia. (Introduced by Elmer L. DeGowin).

Others have described methods for obtaining pressure pulse waves by preoperative left atrial needling in attempts to distinguish predominant mitral stenosis from predominant incompetence. During cardiotomy we have recorded directly left atrial waves from eleven patients with preponderant mitral incompetence and simultaneous left ventricular waves from two of them.

Analytical observations: 1) Left ventricular end-diastolic pressure is significantly lower than simultaneous left atrial pressure; 2) The commonest left atrial waves in predominant mitral incompetence have sharp, jagged upstrokes, climbing to peaks and falling rapidly. Very similar tracings are recorded from some patients with pure mitral stenosis; 3) Two patients with predominant incompetence had greater pulse pressure (associated with low diastolic pressure) than any case of preponderant stenosis; 4) Low diastolic pressure is found in some symptomatic patients with pure mitral stenosis; 5) Plateau-type tracings may be found in either predominant stenosis or incompetence; 6) One patient with predominant incompetence had a sharp upstroke, a relatively flat top and a sharp downstroke. No similar tracing was found in 95 patients with preponderant stenosis; 7) No patient with predominant incompetence showed a well-marked sharp "o" wave following the fall of the "v" wave; 8) One patient with stenosis, who developed severe incompetence during operation immediately after valvuloplasty (which proved fatal 10 days later) showed striking acute changes in the left atrial waves; but, considered on its own merits, this tracing was not diagnostic of predominant mitral incompetence; 9) "A" waves in preponderant incompetence ranged 0 to 4 mm. Hg and in predominant stenosis, 0 to 13.

We have concluded that it is impossible to diagnose predominant mitral incompetence with assurance from analysis of left atrial pressure pulse waves. Therefore, we believe that the clinical risk of blind preoperative needle puncture of the left atrium, by whatever route, is unjustified.

A Study of the Osmometric Behavior of the Human Red Blood Cell. T. F. WILLIAMS, C. C. FORDHAM, and W. HOLLANDER, JR., Chapel Hill, N. C. (Introduced by C. H. Burnett).

Aliquots of whole defibrinated blood were either untreated or treated with 1) addition of solutions of different concentrations of salt or sucrose, and 2) addition of different amounts of dry urea. The osmotic activity of these specimens was estimated by freezing point depression initially and after lysis of the cells induced by freezing.

The osmotic activity (mOsm. per L.) of the hemolyzed specimen (C_H) was less than the unlysed (C_S). The decrease in activity after lysis was least with untreated blood, intermediate in the specimens treated with urea, and greatest in those to which solutions of NaCl or sucrose had been added. In the salt and sucrose studies the loss of activity increased as C_S increased. These data fit the equation: $C_H = 0.9728\ C_S + 4.06$.

The relationship between (C_S–C_H) and the proportion of the total water content of the lysed specimen derived from cells is a curve. These data should have described a straight line if the loss in activity resulted from the admixture of hypotonic cell water with serum water, or the liberation of "bound" water associated with lysis.

The anticipated and observed values for C_S in specimens of blood with known water content and initial activity to which solutions whose water content and activities were known had been added agree within 1 to 2 per cent, but deviations are unidirectional at the various levels and are significant.

The directions of these deviations and the loss of activity with lysis are consistent with Adair's data concerning the relationship between osmotic activity and concentration of hemoglobin. Moreover, when that relationship is applied to these data the discrepancies are considerably diminished or eliminated. The data, therefore, appear to be consistent with the hypothesis that red cells behave as perfect osmometers in a range extending from 210 to 750 mOsm. per L.

Vasodilators in Plasma from Patients with Infections and Altered Nutritional States. CHARLES L. WISSEMAN, JR. and SHELDON E. GREISMAN, Baltimore, Md. (Introduced by Joseph E. Smadel).

Changes in vascular physiology contributing to plasma leakage, hemo-concentration and shock are important in

the pathogenesis of spotted fever and hemorrhagic fever (HF) and also occur in experimental animals inoculated with certain microbial products. The possible occurrence of humoral vasoreactive factors which contribute to the markedly dilated and excessively permeable capillaries of patients with HF was investigated in Korea. Plasma (1.5 to 2 ml.) from acutely ill HF patients, upon intravenous injection into rabbits, caused delayed (1 to 3 minutes) but intense and persistent (5 to 20 minutes) dilatation of the small conjunctival vessels. Similar effects were not usually obtained with convalescent plasma or random plasmas taken from normal persons. The active component was non-dialyzable, unstable on storage at 4° or −70° C. and promptly destroyed by freezing with ether.

Plasmas from normal persons taken post-prandially and also after 48 hours' starvation, but not after 24 hours, exhibited identical vasodilator action. Similar effects were also obtained with some plasmas from patients with a variety of acute febrile illnesses. Thus, the reaction appears dependent upon endogenous material occurring under a number of circumstances. The active component in post-prandial and starvation plasmas possessed properties similar to those listed for HF plasmas. Although certain of its properties suggested a lipoprotein, the factor appeared with the alpha globulins on fractionation and was clearly separated from those lipoproteins which rise at $d = 1.063$ upon ultracentrifugation.

The nature and normal function of the vasodilator substance under discussion remains obscure, but it may be related to the homologous capillary permeability factors of Mackay *et al.* or the heterologous capillary damaging factor of Lake *et al.* The probable importance of this newly recognized phenomenon in man in infectious and nutritional states deserves additional investigation.

The Stressful Interview as an Experimental Tool. Stewart Wolf,* Oklahoma City, Okla.

A stressful life experience is a stimulus which, like contact with bacteria, is not strictly quantifiable in Man. Since the force of the stress derives from the significance of the event and, since significance varies with the shading of attitudes, values, and experiences of each individual, it is unlikely that such stress will ever be described in quantitative terms.

The stressful interview is a prototype of the stressful event which can be introduced artificially in such a way as to satisfy most of Koch's postulates. Its subject matter is selected on the basis of previous knowledge of the patient and his bodily responses during certain events in his life. By applying the stimulus of the interview at a time when the bodily indicators are being measured, and after a suitably recorded control period, the earlier evidence of coincidence can be supplemented by direct experimental testing, *i.e.*, discussion of the event which coincided in time with a flare-up of the bodily disturbance in question. Then an attempt can be made by reassurance to withdraw the stimulus and restore the control situation.

The stressful interview has a special place in the evaluation of pharmacodynamic agents which act on central nervous system and autonomic mechanisms. The stressful interview allows one to test the potency of such agents by opposing them with the kind of force which would be encountered in the patient's daily life.

Representative data from nearly 3,000 stressful interviews applied 1) to help establish the relevance of life experience to a variety of bodily disorders and 2) to evaluate the potency of pharmacodynamic agents which act on central nervous and autonomic mechanisms will be presented in support of this technic as a serviceable and reliable experimental tool.

Peripheral Venoconstriction in Human Congestive Heart Failure. J. Edwin Wood, Julius Litter, and Irwin H. Friedman, Boston, Mass. (Introduced by Robert W. Wilkins).

The purpose of this study was to measure peripheral venous distensibility in congestive heart failure. The measurement was repeated after recompensation. Venous distensibility was also measured in a group of patients with no cardiovascular disease.

Forearm venous distensibility was measured with a water-filled, variable depth volume recording plethysmograph. Prior to each experiment, the *effective* local venous pressure (internal pressure minus external pressure) and the venous volume of the forearm segment were reduced to a low, constant, reproducible value by adjusting the external water pressure on the forearm to a level which exceeded the previously measured natural local venous pressure. With this as a starting point, local effective venous pressure was increased by 30 mm. Hg with a proximal cuff. The resultant forearm volume increase was recorded and converted to cc. per 100 cc. of forearm tissue. This figure was the venous distensibility. A low value indicated peripheral venoconstriction. Results obtained were dependent upon the resistance of the local vein walls to a standard differential pressure. The results were not influenced by the level of central venous pressure *per se*.

Eighteen patients with congestive heart failure associated with elevated venous pressure had a mean venous distensibility of 2.9 cc. per 100 cc., S.D. 0.5 cc. Twelve patients with congestive heart failure but normal venous pressure had a mean venous distensibility of 3.7 cc. per 100 cc., S.D. 0.8 cc. Ten patients with no cardiovascular disease had a mean venous distensibility of 4.5 cc. per 100 cc., S.D. 0.5 cc. Five patients with congestive heart failure were restudied after compensation occurred and venous distensibilities had increased from 2.3 to 3.5 cc., 3.2 to 4.3 cc., 2.1 to 4.4 cc., 2.5 to 3.3 cc. and 2.4 to 3.1 cc. per 100 cc., respectively. These studies indicated that peripheral venoconstriction was present in patients with congestive heart failure. The venoconstriction tended to remit as the patient improved.

Symptomatic Treatment of Subjects with Presumably C.N.S. Arteriosclerosis by the Intravenous Administration of Histamine. Janet Worrell, Rochester, Minn. (Introduced by Bayard T. Horton).

Twenty-one subjects with a presumptive diagnosis of C.N.S. arteriosclerosis were selected for this special

study. Ages ranged from 57 to 85 years. The average age was 65.4 years. Histamine was administered intravenously in either a 1:250,000 dilution or a 1:500,000 dilution for a period of one and one-half hours daily.

Thirteen of the 21 patients (62 per cent) noted marked symptomatic improvement. This group received a mean of 42 daily histamine treatments with the range from 3 to 134. Eight patients (38 per cent) noted no subjective improvement in their symptoms. This group received a mean of 10.5 daily treatments with the range of 2 to 26. Six patients returned for further therapy and three were given treatment at home. Of these nine patients, five again received symptomatic relief.

A patient was considered to have obtained marked symptomatic improvement when vertigo or ataxia had disappeared after treatment and the patient was able to walk unassisted, or when the patient had complete relief from dizziness and headache and improvement in memory or mental alertness. If the patient was only partially relieved of these symptoms and admitted to 50 to 60 per cent improvement, he was considered not improved along with those who had no change in symptoms. Before treatment, eleven (52 per cent) of the patients had vertigo or ataxia so that they could not walk alone; seven (63 per cent) of these had relief of symptoms at the time of discharge.

The significant difference in the mean number of intravenous histamine treatments given the group with marked symptomatic improvement as compared with the group with no improvement would seem to indicate that only prolonged intravenous histamine may be effective in symptomatic treatment of these patients.

Serum Glutamic Oxalacetic Transaminase as an Index of Liver Cell Injury. FELIX WROBLEWSKI and JOHN S. LADUE, New York City, N. Y. (Introduced by Robert F. Watson).

Glutamic oxalacetic transaminase has been found in all human sera studied. This enzyme is present in high concentration in liver as well as other tissues such as heart, kidney, muscle, and brain. Variations in serum transaminase in more than 100 patients with various types of liver diseases will be presented and correlated with liver function tests.

In human as well as experimental animals, injury to liver tissue by carbon tetrachloride results in elevation of serum transaminase activity of 50 to 1000 times normal. The levels remain high for variable periods and fall with or before clinical improvement. The enzyme activity in infectious or homologous serum hepatitis rises 10 to 80 times normal in all patients studied during the first 7 to 10 days of their disease. Transaminase activity was moderately increased (15 per cent to 600 per cent) in patients with metastatic cancer of the liver and this elevation appears to be a fairly sensitive sign of metastatic liver disease. Cirrhotics have variable levels which appear to rise during activation of their disease. The height of transaminase activity and the pattern of serial determinations appear to be worthy of further study in the evaluation of liver disease.

Pathways of Urate Synthesis in Gout. JAMES B. WYNGAARDEN, Bethesda, Md. (Introduced by William P. Chapman).

The present study was designed to compare rates and extents of glycine incorporation into purines of normal and gouty persons, to gain information about biosynthetic intermediates of normal and abnormal urate production. One control (osteoarthritic) and two gouty men were given 5 μc of glycine-1-C^{14} (.13 mg.) orally while on a purine free, low protein diet. Urinary purines were isolated as copper salts, dissolved in HCl, and uric acid separated by reduction of volume and chilling. Residual purines were further purified *via* copper and ammoniacal silver precipitations, and placed on Dowex-50-H^+ column (200 to 400 M, 2.5×12 cm. bed) as hydrochlorides. Separation was achieved by gradient elution with HCl ($0.15 \rightarrow 2.65$ N). Purines were recovered, recrystallized, and counted. In the control, specific activity of urinary urate (day 1) was 151 c.p.m. per mM, whereas in gouty patients values were 488 and 618 c.p.m. per mM. The first gouty patient was clearly an "overproducer," excreting 822 mg. of urate daily, whereas the second had a normal daily output of 400 mg. In the control, specific activities of xanthine were slightly but not significantly lower than those of urate on the first two days, whereas in the gouty subjects initial xanthine specific activities were only $\frac{1}{3}$ to $\frac{1}{2}$ those of corresponding urate samples. These findings suggest that gouty subjects produce urates by two pathways, one of which does not proceed *via* xanthine, and that this latter pathway is responsible for excessive urate production. In the control subject, two routes may also have been present, but findings suggest that the pathway by-passing xanthine may be of minor importance. Specific activity determinations of hypoxanthine, adenine, and 7-methylguanine are currently being made; the presence of isotope in 7-methylguanine establishes for the first time its participation in human purine metabolism.

The Demonstration of Antileukocytic Complement-Fixing Antibodies in the Serum of Patients with Disseminated Lupus Erythematosus. HYMAN J. ZIMMERMAN, PAUL HELLER, and VINCENT YAKULIS, Chicago, Ill. (Introduced by Granville A. Bennett).

The demonstration of the L.E. phenomenon in some patients with penicillin hypersensitivity and of the ability of rabbit antileukocytic serum to induce nucleophagocytosis, to a degree simulating L.E. cells, led to the hypothesis that the L.E. plasma factor might be an antileukocytic antibody. The present study was undertaken in the attempt to test this possibility.

An extract of human leukocytes, from granulocytic leukemia or normal blood was used as antigen. Using a modified Kolmer Technique with this antigen, 400 sera were tested for complement-fixation.

Positive reactions were obtained in 70 per cent of (7 out of 10) patients with disseminated lupus erythematosus and in 80 per cent of (4 out of 5) patients who had received multiple transfusions, and in guinea pigs sensitized to this antigen. Complement-fixation also was

demonstrated in individual patients with chronic agranulocytosis, rheumatoid arthritis, drug hypersensitivity and in two patients receiving penicillin for pneumonia.

All of the patients with disseminated lupus erythematosus, who had positive complement-fixation showed the L.E. cell phenomenon, but there was a poor correlation between the intensity of the L.E. cell phenomenon and of complement-fixation. A positive correlation was demonstrated between the production of nucleophagocytosis by these sera and the sera of patients with the other diseases cited and the ability to fix complement in the presence of the antigen.

In the remainder of the 400 sera tested, the reaction was negative. Sera high in content of anti-A, anti-B and anti-RH antibodies produced no fixation of complement.

These data suggest that antileukocytic antibodies occur in most patients with disseminated lupus erythematosus, in patients who have received multiple blood transfusions, and in a few patients with other diseases.

Effects of Sympathomimetic Amines on Cardiac Rhythmicity and Conduction in Man. PAUL M. ZOLL, ARTHUR J. LINENTHAL, LEONA R. NORMAN, MILTON H. PAUL, and WILLIAM GIBSON, Boston, Mass. (Introduced by S. L. Gargill).

Quantitative comparisons have been made in man of certain specific cardiac effects of several sympathomimetic amines: arousal, acceleration and maintenance of idioventricular pacemakers; excitation of multifocal ventricular activity; and improvement of atrioventricular conduction. Little precise clinical information on these actions is available although these drugs are widely used as cardiac stimulants and vasopressor agents.

Unique opportunities for controlled observations were presented by six patients with complete atrioventricular block and persistent absence of spontaneous ventricular activity. While life was maintained for hours or days by an external electric cardiac pacemaker, these drugs were administered intravenously at graded rates. The relative magnitude, duration, and dose-response relations of these effects were determined by close electrocardiographic observation and were related to blood pressure changes.

Epinephrine, norepinephrine, and isopropylnorepinephrine differed only quantitatively. Epinephrine appeared most effective for the arousal, acceleration, and maintenance of an idioventricular pacemaker; these responses often occurred promptly with doses (4 to 10 micrograms per minute) which did not excite ectopic ventricular activity and which did not affect the blood pressure significantly even in patients with hypertension. The small doses of norepinephrine required to elevate the blood pressure sometimes excited ectopic ventricular activity, which terminated at once when the drug was stopped. Isopropylnorepinephrine sometimes excited multifocal ventricular activity in the same dose required to arouse an idioventricular pacemaker; the effect on the blood pressure, if any, was a depressor one. Careful comparison of the effects of these and other sympathomimetic amines delineated their beneficial and untoward actions and indicated the agent of choice for each of a variety of specific therapeutic purposes.

All these agents often restored some degree of atrioventricular conduction. This effect indicates a functional, in addition to the known structural, basis for atrioventricular block and supports a pharmacologic approach to its treatment.

An Analysis of the Chemical Events and their Interrelationships with Alterations in the ECG During Respiratory Acidosis and Alkalosis. S. B. JOYNER, D. A. DAVIS, D. T. YOUNG, E. CRAIGE and L. G. WELT,* Chapel Hill, North Carolina.

The chemical alterations induced by respiring high concentrations of CO_2, and hyperventilation with room air have been studied in three groups of dogs as follows: 1) simultaneous arterial and central venous blood, 2) simultaneous arterial, peripheral and hepatic venous blood, and 3) arterial blood samples in experiments conducted 48–72 hours following nephrectomy and injection with inulin.

The effects of the initial induction of hypercapnia from the control state are: an increase in pCO_2 with the associated decrease in pH, an increase in the inulin space, and an accession of sodium, potassium, glucose, and phosphorus to the extracellular fluid. The increment of potassium appeared to be derived from the muscles, glucose from the liver (splanchnic area), and phosphorus from both.

Hypocapnia following a period of hypercapnia is associated with: an increase in pH which accompanies the decrease in pCO_2, a further increase in the volume of distribution of inulin, and a further accession of potassium, glucose, and phosphorus to the inulin space. Potassium is lost from the liver but gained by muscle. The liver releases glucose and both liver and muscle release phosphorus.

The alterations associated with the change from hypocapnia to hypercapnia contrast with those which accompany the initial induction of hypercapnia and are as follows: a decrease in the inulin space, and losses of sodium, potassium, glucose, and phosphorus from the extracellular fluid.

The electrocardiographic alterations observed in these studies resemble those seen with hyperkalemia. However, the attempt to correlate the independent chemical events with the degree of electrocardiographic deterioration was unsuccessful, and serves to emphasize the fact that alterations in the ECG, like most other physiologic events, must be a net result which represents a vector derived from many diverse influences.

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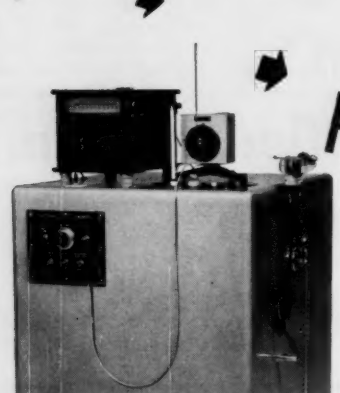

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1. Wood, H. F., and McCarty, M.: Am. J. Med. *17*:768 (Dec.) 1954. 2. Stollerman, G. H., et al.: Am. J. Med. *15*:645 (Nov.) 1953. 3. Rantz, L. A.: Disease-a-Month *1*:18 (Oct.) 1954. 4. Kroop, I. G., and Shackman, N. H.: Proc. Soc. Exper. Biol. & Med. *86*:95 (May) 1954.

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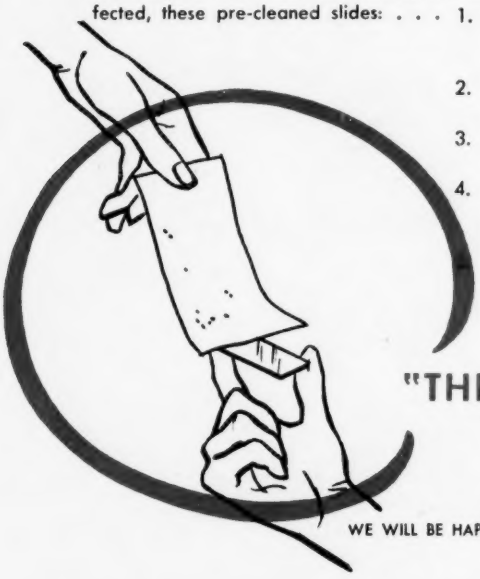

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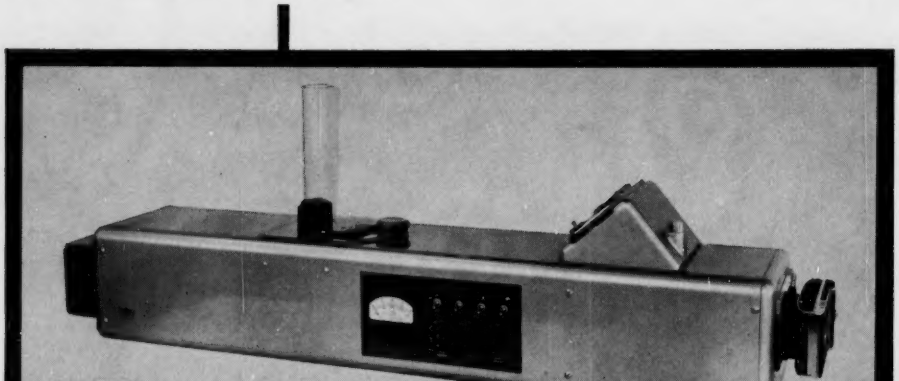

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